

# SCIENTIFIC AGRICULTURE

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# SCIENTIFIC AGRICULTURE

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KELP MEAL IN THE RATION OF GROWING CHICKENS  
AND LAYING HENS<sup>1</sup>T. M. MACINTYRE<sup>2</sup> AND M. H. JENKINS<sup>3</sup>*Dominion Experimental Farm, Nappan, N.S.*

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Dehydrated kelp (seaweed) is sold and used for a variety of purposes in human and animal nutrition. Despite the fact that dehydrated seaweeds have been used for some time as a food for both man and animals (1), comparatively little information is available on the actual food value of this material, or the benefits—if any—derived from its use in poultry rations. Kelp meal is sold as such, and also as an ingredient in proprietary feed supplements. One such supplement is a mixture of kelp meal, fish meal and salts sold under the name of "Manamar". Miller and Bearse (4) report that this mixture prevented rickets in chicks, but that pure kelp meal was not a good source of vitamin D.

Chemically, seaweeds contain a considerable variety of mineral elements and are high in ash, as high as 45 per cent in some species (3). These authors analysed a number of the more common seaweeds of the Maritime Provinces of Canada for moisture, protein, lipid, ash, calcium, phosphorus and iron. The variability in composition of the different species is quite marked and the feeding value of any dried seaweed product would depend on the species of seaweed used. These authors report a protein content as high as 25 per cent for some species and as low as 3.7 per cent for others. Usually about 50 per cent of the dried seaweed, calculated on a dry weight basis, is carbohydrate material. Average analysis for kelp meal runs about 6 per cent protein, 40-50 per cent carbohydrate and 25-35 per cent ash.

The use of dried seaweed as a food has been studied by several workers. Morris (6), in a study on the nutritive value of kelp meal for animal feeding, reports that kelp meal had a definite beneficial effect on the growth of rats. The kelp meal seemed to stimulate the appetite and improve the digestion. Agar and Irish moss fed at levels of 5-10-20 and 30 per cent and 5-10 and 20 per cent, respectively showed good results on the growth of rats according to Nilson and Schaller (7). All levels of agar and Irish moss showed satisfactory results except the 20 per cent level of Irish moss. However, the digestibility of the dry matter in the diet was depressed as the per cent of agar and Irish moss in the diet was increased. The digestibility of agar is given as 28 per cent and Irish moss as 50 per cent. In a survey of the possible uses of kelp, Moore (5) concludes that kelp meal is of little value to animals except the ruminant and that it serves mainly as a conditioner and intestinal lubricant. In a similar study, Delf (2) concludes that seaweeds

<sup>1</sup> Contribution from Poultry Division, Experimental Farms Service, Canada.<sup>2</sup> Research Officer.<sup>3</sup> Technical Officer.



are valuable mainly as a source of bulk and minerals. In a study of the digestibility of seaweed meal (*Laminaria digitata*) using pigs, Sheehy and co-workers (10) found that 67-75 per cent of the organic matter was apparently digested. They point out, however, that part of this rather high nutritive value may be due to increased digestion of the remainder of the ration due to favourable intestinal conditions caused by the presence of the seaweed meal in the ration. The total digestible nutrients of the laminaria in this case was about 50, compared with 20 for potatoes and 40 for hay. In a similar study with sheep, Senior, Collins and Kelly (8) studied three seaweeds, *Fucus*, *Ascophyllum* and *Laminaria*, as sources of energy. The *Fucus* samples contained only very small amounts of digestible organic matter. The *Laminaria* contained more digestible nutrients but varied from season to season, with the best sample being collected in the autumn. The feeding value was comparable with meadow hay. The *Ascophyllum* was about as good as the *Laminaria*. Later work by Sheehy (9) also indicates that different species of seaweed have different nutritive values, but all have a beneficial effect on the physical condition of the intestine, when fed in moderate amounts in the ration.

Few studies appear to have been made on the use of kelp meal in the rations of growing chickens or laying hens. The 1947 and 1948 annual reports of the Scottish Seaweed Research Association make mention of work along this line, but present no conclusions.

Sumita, Kawabota and Fujioka (11) in a study of the influence of kelp meal feed on the iodine content of hens' eggs, found that the feeding of kelp meal increased the iodine content of the eggs. These authors fed kelp meal at the rate of 4 to 10 per cent of the ration. No detrimental effects were noted.

The purpose of the study reported herein was to determine the value of kelp meal in the rations of growing chickens and laying hens. The kelp meal used was supplied through courtesy of the National Sea Products Limited, Halifax, Nova Scotia. The analysis of this kelp meal was as follows:—Moisture, 7.43 per cent; protein (N X 6.25), 6.40; ether extract, 1.55; crude fibre, 9.37; ash, 20.13; calcium, 1.29; phosphorus, 0.12; iodine, 0.26; salt (NaCl), 2.00.

#### EXPERIMENTAL

In this study, three separate experiments (1, 2, 3), were carried out with laying hens and four with growing chickens (4, 5, 6, 7). In the first experiment a ration containing 10 per cent of kelp meal was tested. Two lots of 200 birds each were used. The birds were selected at random from mature Barred Plymouth Rock pullets raised on range under identical conditions, and randomized into 20 pens, with 20 birds in each pen.

One group of 200 birds acted as the control group and received the control or basal ration. The other group of 200 birds was fed 10 per cent of kelp meal as an addition to the basal or control ration in which the salt was reduced by one-half. The composition of the basal or control ration was as follows:—ground oats, 30.0 per cent; ground barley, 22.5; ground wheat, 35.0; wheat bran, 2.5; white-fish meal, 5.0; vita-gras, 2.5; bone meal, 1.5; oyster shell, 1.0; salt, 0.5; fish oil, 0.75; synthetic riboflavin,



$\frac{1}{2}$  gram per ton; manganese sulphate,  $\frac{1}{4}$  pound per ton. No whole grains were fed, but the mash was kept before the birds at all times. Coarsely ground oyster shells were fed ad lib. in hoppers.

The birds were maintained under treatment for 252 days beginning in November, 1948. A daily record was maintained of egg production and egg weight per pen. Feed and shell consumption per pen was also recorded, and individual egg records were maintained for four pens on each treatment for the first 112 days of the trial. In addition, the individual body weight of the birds was recorded at intervals during the experiment. Egg-shell strength was measured by the specific gravity method.

In the second experiment 96 birds were started in each group. The birds were housed in individual cages of laying batteries and 2.5 per cent by weight of kelp meal was added to the basal ration. The birds were fed individually and individual egg and feed records were maintained. The birds were kept on the experimental treatment for 224 days beginning in December, 1949. A daily record of egg production and egg weight was maintained. The specific gravity of a representative number of eggs from each bird was recorded.

The third project was carried out in co-operation with a commercial egg producer. The birds were handled as a commercial laying flock and were all housed in the same building. All pens were treated alike, except

TABLE 1.—EFFECT OF FEEDING 10 PER CENT KELP MEAL IN THE RATION OF LAYING HENS ON EGG PRODUCTION, FEED CONSUMPTION, EGG WEIGHT, BODY WEIGHT AND SPECIFIC GRAVITY OF EGGS

Criteria of comparison	Means of 10 pens		Diff. req. for sig.
	Control	Kelp	
Number of birds per pen	20	20	—
Total bird days per pen	4765	4760	—
Egg production—			
Number of eggs per pen	2678	2692	—
Number of eggs per bird per day	0.56	0.56	—
Pounds of eggs per bird per day	0.075	0.075	—
Individual egg production <sup>1</sup>	49 $\pm$ 21 <sup>2</sup>	46 $\pm$ 23	—
Feed efficiency for egg production—			
Number of eggs per pound of feed	1.96	1.78	0.10
Pounds of eggs per pound of feed	0.26	0.23	0.01
Feed consumption—			
Pounds of feed per bird per day	0.29	0.32	0.01
Pounds of shell per bird per day	0.011	0.012	—
Body weight of bird (pounds)—			
Average initial body weight	5.56	5.64	—
Average final body weight	6.82	6.71	—
Average egg weight <sup>3</sup> (gm.)	62.05 $\pm$ 2.58	61.56 $\pm$ 3.47	—
Average specific gravity of eggs <sup>4</sup>	1.0853 $\pm$ 0026	1.0846 $\pm$ 0042	—

<sup>1</sup> Average egg production of the 80 hens trapnested during the first 112 days.

<sup>2</sup> Standard deviation.

<sup>3</sup> Mean of all eggs weighed for the 80 hens being trapnested.

<sup>4</sup> Mean of all eggs tested for the 80 hens being trapnested.

TABLE 2.—EFFECT OF FEEDING 2.5 PER CENT KELP MEAL IN THE RATION OF LAYING HENS HOUSED IN INDIVIDUAL LAYING BATTERIES

Criteria of comparison	Control	Kelp
Number of birds started	96	96
Total bird days	20440	20664
Egg production—		
Total number of eggs	11932	12114
Number of eggs per bird per day	0.58	0.59
Pounds of eggs per bird per day	0.079	0.079
Individual egg production <sup>2</sup>	133 ± 32 <sup>3</sup>	134 ± 28
Feed efficiency for egg production—		
Number of eggs per pound of feed	1.77	1.75
Pounds of eggs per pound of feed	0.24	0.24
Feed consumption—		
Pounds of feed per bird per day	0.33	0.33
Pounds of shell per bird per day <sup>1</sup>	0.017	0.017
Body weight of bird (pounds)—		
Average initial body weight	5.62	5.43
Average final body weight	6.41	6.47
Average egg weight (gm.)	61.52	61.51
Average specific gravity of eggs	1.0856 ± 0038	1.0861 ± 0035

<sup>1</sup> Oyster shell added to mash at rate of 5 per cent.<sup>2</sup> Average number of eggs per hen surviving for entire experiment.<sup>3</sup> Standard deviation.

TABLE 3.—THE EFFECT OF FEEDING 2.5 PER CENT OF KELP MEAL IN THE RATION OF A COMMERCIAL FLOCK OF LAYING HENS

Criteria of comparison	Control	Kelp
Total number of birds started	994	998
Per cent mortality and culls	21	20
Egg production per bird—		
Hen-housed basis	151	152
Survival basis	192	191
Hatchability—		
Number of eggs set	1382	1176
Number of eggs hatched	1098	940
Per cent hatched	79.4	79.9
Penetrometer reading of shell strength	12.88	12.88

that 3 pens were fed pellets containing 15 per cent kelp meal at a rate which allowed the birds about 2.5 per cent of kelp meal of the total feed consumed. The three control pens were fed a similar quantity of pellets containing no kelp meal. Records of egg production, feed consumption and shell strength were maintained over a 10-month period. In this experiment shell strength was measured by penetrometer readings rather than by specific gravity measurements. Penetrometer readings measure the force required to puncture the large end of the egg-shell with a small sharp pointed needle. Hatchability data were also recorded.



TABLE 4.—PER CENT BASAL RATIOMS

Ingredients	Experiments	
	4 and 7	5 and 6
	%	%
Yellow corn meal	29.5	23.6
Ground oats	—	19.7
Wheat shorts	24.6	19.7
Wheat middlings	24.6	19.7
Alfalfa leaf meal	6.1	5.0
Whitefish meal	6.1	5.0
Soybean meal	4.0	3.0
Dried buttermilk	2.4	2.0
Bone meal	1.2	1.0
Ground limestone	1.2	1.0
Cod liver oil (1500 A 400 D)	0.3	0.3
Total	100.0*	100.0*

\* These basal rations also contained 7 gm. manganese sulphate and 0.2 gm. riboflavin per 100 pounds.

Four experiments were carried out with growing chickens, using 2.5, 5, 10, and 20 per cent kelp meal to replace ground oats in the ration and as a straight addition to an otherwise balanced ration. The composition of the basal rations are shown in Table 4. Table 5 shows the composition and analysis of the diets used in the four experiments. New Hampshire, Barred Plymouth Rock crossbred male chicks were used. They were started when one day old and continued on the experimental diets for six weeks. There were three groups of chicks on each treatment. The number of chicks in each group is shown in Table 6. The groups were randomized, into three, five deck electrically heated battery brooders. The kelp meal was fed in all cases as received from the manufacturer, except in experiment 7, treatment 3, where the kelp meal was washed several times with cold water. This washing seemed to have little effect except to remove some of the colloidal material.

## RESULTS AND DISCUSSION

### *Laying Hens*

Kelp meal fed at the rate of 2.5 and 10 per cent of the ration had little effect on the performance of laying hens. (Tables 1, 2, 3). The only significant differences appear in experiment 1, where the amount of feed consumed by the birds fed the ration containing 10 per cent of kelp meal is significantly greater than the amount of feed consumed by the control group (Table 1). In this experiment the number and pounds of eggs produced per pound of feed are significantly greater for the control group. Other important criteria compared, such as egg weight, egg-shell strength, and body weight of birds all show non-significant differences between the treated and control groups for the three experiments. Aside from the fact that the droppings from the birds fed the 10 per cent level of kelp meal were noticeably moist, no adverse effects of any kind were observed. The 2.5 per cent of kelp meal had no noticeable laxative effect, but at the 10 per cent level, the droppings were so damp that it was necessary to clean the pens out quite frequently. Liveability was not affected by the presence

TABLE 5.—COMPOSITION AND CHEMICAL ANALYSIS OF RATIONS

Experiment <sup>1</sup>	4					5					6			7		
	Treatments <sup>2</sup>					Treatments					Treatments			Treatments		
	1	2	3	4	5	1	2	3	4	5	1	2	3	1	2	3
Per cent ingredient	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Basal ration	79.5	79.6	79.7	79.9	80.0	99.5	97.1	94.7	89.9	80.0	99.5	93.3	87.2	79.5	77.5	77.5
Ground oats	20.0	17.5	15.0	10.0	—	—	—	—	—	—	—	—	—	20.0	9.7	9.7
Iodized salt	0.5	0.4	0.3	0.1	—	0.5	0.4	0.3	0.1	—	0.5	0.25	0.1	0.5	0.1	0.1
Kelp meal	—	2.5	5.0	10.0	20.0	—	2.5	5.0	10.0	20.0	—	5.0	9.7	—	9.7	9.7 <sup>3</sup>
Soybean meal	—	—	—	—	—	—	—	—	—	—	—	1.45	3.0	—	3.0	3.0
Per cent <sup>4</sup>																
Moisture	13.4	13.6	13.0	13.8	13.4	13.2	13.0	13.1	13.3	12.9	10.9	10.8	11.7	12.0	12.4	12.6
Ash	6.3	6.6	6.8	7.6	9.3	7.5	7.9	7.9	8.5	10.1	7.0	7.6	8.1	6.4	8.2	8.1
Crude protein	20.5	20.0	20.0	19.8	19.0	21.0	20.5	20.2	19.5	17.5	20.0	19.4	19.4	20.3	20.4	20.9
Crude fat	6.3	5.4	5.1	5.1	5.0	5.9	6.2	6.4	5.7	5.1	6.0	6.0	4.9	4.9	4.9	4.5
N. free extract	46.2	46.2	48.1	47.0	47.2	44.1	43.7	43.9	44.7	46.2	49.1	48.7	48.3	50.6	46.5	45.7
Crude fibre	7.3	8.2	7.0	6.7	6.1	8.3	8.7	8.5	8.3	8.2	7.0	7.5	7.6	5.8	7.6	8.2
Calcium	1.48	1.35	1.40	1.28	1.46	1.34	1.35	1.40	1.34	1.40	1.60	1.67	1.67	1.69	2.02	2.04
Phosphorus	0.59	0.63	0.65	0.58	0.55	0.58	0.57	0.58	0.55	0.49	0.69	0.71	0.53	0.62	0.60	0.57

<sup>1</sup> Experiment 4. Kelp meal fed as a substitute for ground oats in the ration.<sup>2</sup> Experiment 5. Kelp meal fed as an addition to a balanced ration.<sup>3</sup> Experiment 6. Kelp meal fed as an addition to a balanced ration with soybean meal added to compensate for the calculated protein in the kelp meal.<sup>4</sup> Experiment 7. Kelp meal fed as a substitute for ground oats in the ration with soybean meal added to compensate for the calculated protein in the kelp meal.<sup>5</sup> Treatment 1 is the control treatment in all four experiments. Other treatments differ from the control by the substitution and/or addition of kelp meal and soybean meal as indicated.<sup>6</sup> Kelp meal contains about 2 per cent of salt and an adjustment in the salt added to the ration was made to account for this.<sup>7</sup> Kelp meal washed with water.<sup>8</sup> Actual chemical analysis.



of kelp meal in the ration and in experiment 3 (Table 3), a test on the hatchability of the eggs showed no significant difference between the treated and control groups.

From the results of these experiments it would appear that from 2.5 to 10 per cent of kelp meal may be safely added to a balanced ration without upsetting egg production, although no beneficial effects accrue from the feeding of this material.

### *Growing Chickens*

When chickens were fed a ration containing 2.5, 5, 10 and 20 per cent kelp meal, growth was generally depressed as compared to the control rations containing no kelp meal. In experiment 4, where kelp meal was substituted for ground oats in the ration, at 2.5, 5 and 10 per cent levels, the differences in the mean final body weights between the control and the groups receiving 2.5, 5 and 10 per cent of kelp meal are non-significant, but when 20 per cent of kelp meal was added to the ration, growth was significantly lower than for any of the other groups. However, with the exception of the 2.5 per cent level, feed efficiency was greatly impaired by the substitution of kelp meal for ground oats in the ration (Table 6).

In experiment 5 (Table 6), when kelp meal was added at the rate of 2.5, 5, 10 and 20 per cent to a balanced ration, significantly decreased growth rates were observed in all cases where kelp meal was fed when

TABLE 6.—THE EFFECT OF FEEDING DRIED GROUND SEAWEED UPON THE GROWTH OF CHICKS

Experi- ment <sup>7</sup>	Treat- ment <sup>7</sup>	Per cent kelp in ration	Number of chicks per group <sup>5</sup>	Mortality	Average feed con- sumption, gm.	Average final body weight, gm.	Gm. feed per gm. body weight
				%			
4	1	—	22	6.1	1191	<sup>1</sup> 472 ± 71 <sup>6</sup>	2.52
	2	2.5		6.1	1198	473 ± 64	2.53
	3	5.0		3.0	1208	460 ± 63	2.63
	4	10.0		13.6	1274	460 ± 79	2.77
	5	20.0		12.1	1227	400 ± 79	3.07
5	1	—	27	8.6	1268	<sup>2</sup> 497 ± 75	2.55
	2	2.5		3.7	1243	462 ± 73	2.69
	3	5.0		1.2	1241	426 ± 58	2.91
	4	10.0		7.4	1297	461 ± 65	2.81
	5	20.0		11.1	1300	388 ± 79	3.35
6	1	—	20	5.0	1419	<sup>3</sup> 533 ± 68	2.66
	2	5.0		8.3	1456	541 ± 72	2.69
	3	9.7		8.3	1486	527 ± 84	2.82
7	1	—	20	11.7	1202	<sup>4</sup> 448 ± 88	2.68
	2	9.7		11.7	1200	441 ± 82	2.72
	3	9.7 (washed)		13.3	1222	444 ± 90	2.75

<sup>1</sup> Least significant difference at 5 per cent level = 26 gm.

1 per cent level = 35 gm.

<sup>2</sup> Least significant difference at 5 per cent level = 22 gm.

1 per cent level = 29 gm.

<sup>3</sup> No significant difference.

<sup>4</sup> No significant difference.

<sup>5</sup> Three groups on each treatment.

<sup>6</sup> Standard deviation.

<sup>7</sup> See footnotes 1 and 2 to Table 5.

compared with the control ration which contained no kelp meal, and feed efficiency was correspondingly impaired. In this experiment the chickens receiving 10 per cent of kelp meal showed significantly better gains than the chickens receiving 5 per cent of kelp meal. The reason for this is not apparent, but the poor growth of the chicks on kelp meal in experiment 5, as compared with experiment 4, may be due to the greater diluting effect of the kelp meal on the ration in this case.

In experiment 6 there are no significant differences between the mean final body weights for any of the treatments. In this experiment the kelp meal was added to the ration at the rate of 5 and 9.7 per cent, and 1.45 and 3 per cent (respectively) of soybean meal was added to the rations to make up for any lack of protein due to the protein in the kelp meal being unavailable to the chick. While the differences in the final weights are non-significant, the feed efficiency at the 9.7 per cent level of kelp meal is not as good as the control.

In experiment 7 where 9.7 per cent of kelp meal (unwashed and washed), was substituted for a similar quantity of ground oats in the ration, and additional soybean meal added, there are no significant differences in rate of growth as indicated by mean final body weight. This is true for both washed and unwashed kelp meal. This would indicate that chickens will tolerate up to 10 per cent of kelp meal in the diet when the kelp meal is substituted for ground oats, or as a straight addition to a balanced ration, if soybean meal is added to compensate for the protein of the kelp meal, but that feed efficiency is impaired by the addition of kelp meal.

The kelp meal did not appear to have any adverse effect on the general health of the birds except when fed at the 20 per cent level. Chicks receiving 20 per cent of kelp meal in the ration looked very unthrifty as compared with the controls. The kelp meal was quite laxative at and above the 10 per cent level, and mortality was greater when higher levels of kelp meal were fed.

#### SUMMARY

(1). Several experiments were conducted to study the effects of feeding kelp meal to growing chickens and laying hens.

(2). Ten per cent of kelp meal in the ration of laying hens caused increased feed consumption and damp droppings resulted, but 2.5 per cent had no measurable effects.

(3). Kelp meal had no effect on mortality, egg production, egg-shell strength, hatchability, or body weight.

(4). Growing chickens will tolerate up to 10 per cent of kelp meal in the ration, as a substitute for ground oats or as an addition to a balanced ration if soybean meal is added to compensate for the calculated protein in the kelp meal.

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# THE EFFECTS OF SEASONAL AND CULTURAL VARIATIONS ON MATURITY OF WOODY PLANTS COMMONLY GROWN ON THE CANADIAN PRAIRIES<sup>1</sup>

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## INTRODUCTION

According to Meyer and Anderson (4) all plant tissues pass through the same morphological stages of growth such as cell division, cell enlargement and cell maturation. In addition to morphological and various biochemical changes that accompany maturity of tissues, this final phase of growth is also characterized by a proportionate decrease in water content as compared to the cell division and cell enlargement phases. Chandler (1) stressed maturity by stating that it is the most important factor affecting the hardness of plant tissues. According to Macoun (3) and Shutt (5), killing back of twigs is an indication of inherent tenderness of the variety or of immaturity of wood. From their work with apples these authors concluded that those trees whose new growth contains the largest percentage of water as winter approaches are in all probability the most tender. Hardiness was considered by Shutt (5) to be more than an inherited tendency but rather a quality largely influenced by cultural practices such as soil condition with respect to moisture and temperature in the late summer and autumn months. According to Shutt (5) "By early arresting vegetative growth and ripening of the new wood by artificial means, such as pruning and cover crops or by a dry and cold autumn, varieties now considered tender might prove hardy."

In the light of the above findings it was considered of practical importance to study the effects of seasonal variations and certain cultural practices on water content of woody twigs during winter, as an index of relative maturity and subsequent winter hardiness.

## EXPERIMENTAL PROCEDURE

The study reported in this paper was carried on during winters of 1950, 1951 and 1952. Mature twigs were taken from 19 kinds of vigorous trees, ranging in age mainly from 10 to 15 years and representative of species and varieties grown on the Canadian Prairies for windbreak or ornamental purposes and for fruit production. Plants studied were as follows:

### *Windbreak species—*

- Caragana, *Caragana arborescens* (Lam.)
- Green ash, *Fraxinus pennsylvanica* var. *lanceolata* (Sarg.)
- American elm, *Ulmus americana* (L.)
- Boxelder, *Acer Negundo* (L.)
- Golden willow, *Salix alba* (L.)
- Cottonwood, *Populus deltoides* (Marsh.)

### *Ornamental species—*

- European larch, *Larix decidua* (Miller)
- Russian olive, *Elaeagnus angustifolia* (L.)
- Soft maple, *Acer saccharinum* (L.)
- Chinese elm, *Ulmus pumila* (L.)

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*Malus spp.* (S. F. Gray)—

- Columbia—crab apple
- Amur —crab apple
- Robin —crab apple
- Rescue —crab apple hybrid
- Wealthy —apple

*Prunus spp.* (L.)—

- Aitkin —plum
- Pembina —plum hybrid
- Morden 118—plum × sand cherry hybrid
- Nanking —cherry

The same trees were used throughout the study. Each tree was represented by about 10 to 12 twigs taken at breast-height and around its whole perimeter. The twigs were cut into sections to fill four desiccation boxes, each containing two or three twigs, and were dried in a thermostatically controlled electric oven for 48 hours at 101° C. The loss of water after oven-drying was determined as per cent of fresh weight. This water content of twigs was used as the criterion of their maturity.

This study was conducted to determine the effect of mulching and wider spacing than the usual recommended 4' × 4' on degree of maturity of caragana, ash, elm, boxelder and cottonwood. The effects of certain seasonal variations on maturity of twigs of 19 horticultural plants collected during winters of 1950, 1951 and 1952 were also compared. The differences in the recorded weather data for these seasons are shown in Table 1. The significance of these effects was determined by statistical methods given by Snedecor (6). The calculated values to indicate the degree of association or least significant difference are presented with the basic data.

TABLE 1.—WEATHER DATA FOR GIVEN PERIODS PRIOR TO COLLECTION OF TWIGS

Weather data	Seasons					
	1949		1950		1951	
	May 24 to Sept. 13, 1949 ( <sup>1</sup> )	Sept. 14 to Jan. 12, 1950 ( <sup>2</sup> )	May 9 to Oct. 1, 1950 ( <sup>1</sup> )	Oct. 2 to Jan. 25, 1951 ( <sup>2</sup> )	April 25 to Sept. 23, 1951 ( <sup>1</sup> )	Sept. 24 to Jan. 4, 1952 ( <sup>2</sup> )
Duration in days	113	121	146	116	152	103
Temperatures in ° F.—						
Mean	60.0	24.8	58.6	17.7	57.9	20.2
Maxima	100.0	82.0	97.0	68.0	96.0	72.0
Minima	29.0	-27.0	31.0	-22.0	29.0	-27.0
Number of days with—						
Max. temp. at optimum ( <sup>3</sup> )	34	—	38	—	23	—
Max. temp. below optimum	64	—	103	—	124	—
Max. temp. above optimum	15	—	5	—	5	—
Max. temp. above 41° F. ( <sup>3</sup> )	—	58	—	29	—	27
Precipitation in inches	6.7	2.1	9.5	1.1	11.4	2.4

(<sup>1</sup>) Frost-free period, i.e. above 29° F. (inclusive), which approximates the growing season.

(<sup>2</sup>) Period between the first killing frost in fall and date of collection mature twigs in winter.

(<sup>3</sup>) According to Meyer and Anderson (4), the optimum temperature range for plant growth is 78°-86° F. Growth may continue at temperatures above 41° F.

The data in Table 1 indicate that the 1949 growing season was somewhat shorter, and distinctly warmer and drier than the 1950 or 1951 season. The 1950 season was slightly warmer and drier by about 2 inches of precipitation as compared to the 1951 season.

Studies of available soil moisture by desiccation method (2) indicated that this was reduced after a dry spell to approximately wilting percentage on September 8, 1949; September 7, 1950; and August 6, 1951. Following these dates the amount of fall precipitation in the form of rain was 1.86, 1.53 and 8.08 (4.77 in Sept. and Oct.) inches, respectively. It is apparent that approximately three additional inches of water were stored in the soil

TABLE 2.—WATER CONTENT, EXPRESSED AS PER CENT OF FRESH WEIGHT, OF MATURE TWIGS OF CERTAIN WOODY PLANTS DETERMINED DURING THE WINTERS OF 1950, 1951 AND 1952. ALL DATA SHOWN ARE AVERAGES OF FOUR SAMPLES

Plants	Dates			Mean
	January 12, 1950	January 25, 1951	January 4, 1952	
<i>Windbreak spp.</i> —				
Caragana	45.8	47.1	48.3	47.1
Green ash	34.8	36.5	35.2	35.5
American elm	45.9	46.1	45.3	45.8
Boxelder	50.2	50.8	54.5	51.8
Golden willow	51.9	52.3	51.0	51.7
Cottonwood	51.3	47.5	51.0	49.9
<i>Ornamental spp.</i> —				
European larch	48.4	51.1	52.3	50.6
Russian olive	46.3	47.6	46.1	46.7
Soft maple	48.6	46.2	54.8	49.9
Chinese elm	47.4	50.2	48.8	48.8
<i>Malus spp.</i> —				
Columbia	48.1	48.2	50.4	48.9
Amur	45.1	45.8	47.5	46.1
Robin	49.4	47.7	47.8	48.3
Rescue	49.0	50.1	50.3	49.8
Wealthy	48.0	47.3	49.2	48.2
<i>Prunus spp.</i> —				
Aitkin	43.1	44.1	44.4	43.9
Pembina	47.0	48.2	45.9	47.0
Morden 118	45.4	45.5	46.8	45.9
Nanking cherry	41.6	43.1	45.3	43.3
Mean	46.7	47.1	48.1	47.3
Difference necessary for significance			At P .05	
Between means of plants			2.5	
Between means of seasons			1.0	
Between individual trees			4.3	

Degree of association ( $r$ ) between water content of mature twigs of 19 woody plants in:  
 1950 and 1951 = 0.906 (\*\*)  
 1950 and 1952 = 0.879 (\*\*)  
 1951 and 1952 = 0.814 (\*\*)



in the fall of 1951 as compared to 1950 and 1949. According to Staple and Lehane (7) in 1951 this would moisten to normal capacity a depth of 32 inches of the clay loam soil in which the trees were grown, as compared to about 12 inches in 1950 and 1949.

It is apparent from the above that the 1949, and to a somewhat lesser extent the 1950 seasons were more conducive to "early arresting of vegetative growth and better ripening of new wood" (5), than the 1951 season.

### EXPERIMENTAL RESULTS

Data on water content of mature twigs studied during the winters of 1950, 1951 and 1952 are presented in Table 2.

The analysed data in this table revealed highly significant associations between the water content of twigs of 19 plants for three successive winters. This indicated a certain constancy in these woody plants with respect to the water content of mature twigs that has not been greatly influenced by the seasonal variations described in this study. The effect of 1949 and 1950 seasons, which favoured better maturity of twigs as compared to 1951, was indicated to a certain extent by caragana, boxelder, European larch, soft maple, Columbia, Amur, Wealthy, Morden 118, and Nanking cherry. Of these, only soft maple and boxelder showed the differences necessary for significance. The three inches of additional fall precipitation that fell in 1951 did not affect the water content of twigs of the remaining 10 kinds of trees that were selected for this study.

Table 3 presents the results of the analysed data on effects of mulching, and wider spacing than the usual recommended 4'  $\times$  4', on the water content of mature twigs of several species commonly used for windbreak purposes on the prairies.

The analysed data of Table 3 failed to present conclusive evidence that the practice of wider spacing than the usual 4'  $\times$  4' or mulching affected greatly the water content of mature twigs of the several selected windbreak species. No significant differences in water content resulted from the four cultural treatments for ash, boxelder and cottonwood. In caragana the wider spacing of 16'  $\times$  16' and mulching resulted in greater maturity of twigs, whereas in elm this wider spacing had no effect and mulching resulted in greater immaturity of twigs. The data of Table 3 also indicated a pronounced effect of 1949 and 1950 seasons as compared to 1951 on maturity of twigs of caragana and boxelder which was revealed by data in Table 2.

### DISCUSSION

The data obtained from this exploratory study indicated that the water content of mature twigs is a persistent physiological expression in woody plants that cannot be easily modified by seasonal variations or cultural practices described in this study. An association between lower water content, i.e. greater maturity, and better winter survival was indicated in a previous study (8) and also by the work of others (1, 3, 5). This would

TABLE 3.—WATER CONTENT, EXPRESSED AS PER CENT OF FRESH WEIGHT, OF MATURE TWIGS OF CERTAIN WOODY PLANTS GROWN UNDER FOUR DIFFERENT CULTURAL METHODS, DETERMINED DURING THE WINTERS OF 1950, 1951, AND 1952. ALL DATA SHOWN ARE AVERAGES OF FOUR SAMPLES

	Dates	Cultural methods				Mean
		4' × 4' and clean cultivation	8' × 8' and clean cultivation	16' × 16' and clean cultivation	4' × 4' with mulch	
Caragana	Jan. 12/50	45.8	45.4	45.0	45.5	45.4
	Jan. 25/51	47.1	46.5	45.6	45.7	46.2
	Jan. 4/52	48.3	48.0	47.6	48.1	48.0
	Mean	47.1	46.6	46.1	46.4	46.5
Green ash	Jan. 12/50	34.8	36.8	35.6	35.0	35.5
	Jan. 25/51	36.5	35.1	37.4	34.7	35.9
	Jan. 4/51	35.2	34.2	34.9	35.5	35.0
	Mean	35.5	35.4	36.0	35.1	35.5
American elm	Jan. 12/50	45.9	43.8	45.7	48.0	45.9
	Jan. 25/51	46.1	45.1	45.8	48.5	46.4
	Jan. 4/52	45.3	44.9	43.5	48.6	45.6
	Mean	45.8	44.6	45.0	48.4	46.0
Boxelder	Jan. 12/50	50.2	46.7	45.6	50.3	48.2
	Jan. 25/51	50.8	50.0	49.5	52.5	50.7
	Jan. 4/52	54.5	56.9	56.3	54.2	55.5
	Mean	51.8	51.2	50.6	52.3	51.5
Cottonwood	Jan. 12/50	51.3	46.5	47.6	49.2	48.7
	Jan. 25/51	47.5	48.2	49.9	51.6	49.4
	Mean	49.4	47.3	48.8	50.4	49.0

Difference necessary for significance between means at P .05	Species				
	Caragana	Ash	Elm	Boxelder	Cotton- wood
Cultural methods	0.6	—	1.5	—	—
Seasons	0.5	—	—	3.3	—

suggest the practicability of careful testing and selecting plants possessing the desired inherent trait in preference to cultural treatments, as a means of increasing hardiness of plants on the prairies. The existence of such plants within the various groups was revealed by this study (e.g. ash, Amur, Aitkin, Nanking cherry).

This suggests further work, with an artificially controlled freezing-unit, on the relation of water content at maturity to cold resistance of plants. The practical value of establishing levels of water content for various woody plants as indicators of their relative winter survival on the prairies is self-evident, and further investigational work along these lines is planned.



## SUMMARY

Exploration studies were conducted during the winters of 1950, 1951 and 1952 to investigate the relative effects of seasonal variations and several cultural treatments on the water content of mature twigs of 19 plants commonly grown on the Canadian prairies for windbreak purposes and fruit production. Obtained data revealed the following:

1. The water content of mature twigs of woody plants is an inherent trait that cannot be easily modified by the cultural treatments or seasonal variations considered in this study.
2. In view of the concept favoured by modern workers that greater wood maturity (i.e. relatively lower water content) is a factor in winter survival, selecting plants which possess the desired trait is suggested as a more practical means of increasing the number of hardy plants on the prairies than resorting to the cultural treatments described in this study.
3. The study revealed the existence of several such plants and further intensive work to determine the importance of water content at autumn maturity as a major criterion of hardiness of woody plants is being contemplated as the results of these exploratory investigations.

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# OBSERVATIONS ON A ROOT AND STEM ROT OF SOYBEANS NEW TO ONTARIO, CAUSED BY *PYTHIUM ULTIMUM* TROW<sup>1</sup>

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On July 10, 1951, in the course of a mid-season survey of commercial stands of soybeans in Kent country, Ontario, the attention of the writers was attracted to dead and dying plants in a field of the variety Hawkeye. These plants, occurring singly or in small groups, were variously scattered throughout the field, and it was estimated that they might aggregate 2 per cent of the total stand. The discovery was of more than ordinary interest because, for one reason, mortality of plants at that particular time of the season was unusual and, for another, the symptoms exhibited by the diseased plants were not recognized as having been encountered previously. On July 11, specimens of the variety Lincoln showing exactly the same symptom-picture were brought to the laboratory by a grower from Essex county. On the same day, search in the laboratory experimental plots revealed the presence of similarly affected plants representing several different varieties. Isolations from specimens from the three different sources yielded pure cultures of an apparently identical *Pythium* species. Prior to this time no *Pythium* species had ever been reported as occurring on this host in Canada. The presence of the fungus having been established here, it seemed advisable to gain some further information, not only about the organism itself, but also about its potential pathogenic capabilities as related to the crop concerned.

## LITERATURE REVIEW

Almost without exception, information regarding *Pythium* spp. as causal agents of disease in soybeans has been furnished by investigators in the United States. As early as 1924, *P. debaryanum* Hesse was mentioned by Wolf and Lehman (19) as the cause of a root disease of soybeans in North Carolina. In 1926, Lehman and Wolf (8) described the symptoms of the disease and the morphology of the fungus. In 1942, Sprague (16) reported that, in North Dakota, *P. debaryanum* was frequently isolated from soybean seeds and, in 1944, Melhus *et al.* (12) stated that this species was one of the organisms most commonly isolated from decayed roots of the same host in Iowa. According to McLaughlin (11), in 1946, *P. debaryanum* caused damping-off of soybeans in Oklahoma and, in the same year, Porter (15) found the species to be a factor in inducing "baldhead" seedlings. Other investigators in the United States have mentioned unidentified species of *Pythium* in association with damping-off and blight of soybean seedlings (17, 3), with a wilting and drying of plants (4), with root necrosis (5, 6), with root and neck rot (10), and with root and stem rots (7). In 1948, Ling (9) includes a *Pythium* sp. in a list of 20 fungi

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parasitic to soybean in Szechwan, China. The latter reference and the present paper constitute the only records of the occurrence of species of *Pythium* parasitic to the soybean outside of the United States.

### DESCRIPTION OF THE DISEASE

Although primarily a rootrot, the disease does, nevertheless, also attack the stems of many affected plants. The effects on the stems are serious enough and the symptoms are clearly enough defined to justify for the disease the broader appellation, stem and rootrot.

What mainly attracted attention to diseased plants in the field in July, 1951, was their dwarfed and either wilted or necrotic condition. Many plants, already dead for some time, had become dry and brown and contrasted sharply with surrounding healthy plants. Other more recently affected plants were in a wilting condition, and it was on these that symptoms of greater diagnostic value were observed. The stems of such plants showed a grayish-brown discoloration which, starting at the ground level, extended upwards for varying distances on the stems. In not a few plants these discoloured areas were more or less covered with a visible fungus growth. In addition to the grayish-brown discoloration, there often extended above the latter, sometimes in streaks, a reddish-brown discoloration.

When a more severely diseased plant was pulled from the soil, usually all that remained of the root system was the main axial root. If a plant was handled more carefully and the soil adhering to the roots was removed in water, the cortical tissues of the larger roots were often found to be so badly disintegrated that they sloughed off easily and left the central woody cylinder exposed. In general, affected plants were marked by a paucity of finer absorbing laterals.

From an examination of a considerable number of affected plants, the impression was gained that infection had taken place at or about the ground level and had later spread upwards in the stem and downwards in the root tissues. In the field in 1951, no new infection of plants was observed to take place after about July 15 and, by that time, most of the plants that had been infected earlier had died.

### ISOLATIONS FROM DISEASED PLANTS

In July, 1951, isolations were made from the lower stem region of a total of 15 diseased plants, 5 being selected to represent each of the three different sources mentioned in the introductory paragraph. From areas freshly exposed by removal of the outermost tissues, small bits of tissue were excised and transferred to poured plates of slightly acidified potato dextrose agar (1 drop 10 per cent lactic acid per 15 ml. of medium). Eight such plantings (4 per plate) were made from each stem. As soon as possible after fungal growth appeared, hyphal tips were cut out and transferred to p.d.a. tubed slants.

Of the total of 120 tissue plantings from the 15 different stems, 102 (= 85 per cent) yielded cultures of *Pythium* which, macroscopically at least, appeared to be identical. Most of the cultures were pure, except for the presence of bacteria. For later work, the latter were eliminated by the method described by Ark and Dickey (2) in 1950.

## INFECTION EXPERIMENTS

*Preliminary Greenhouse Tests*

On July 30, a cornmeal-sand mixture in Erlenmeyer flasks, prepared according to the formula of Miller (14), was inoculated with each of two isolates of *Pythium*, one obtained from Essex county plants, the other from Kent county plants, the two isolates being hereinafter referred to as E and K, respectively. On August 10, four flats of steamed soil were infested with the E, and another four with the K isolate, by adding 450 grams of the cornmeal-sand inoculum to the 850 cu. in. of soil with which each flat was filled. Two of the E-infested flats were planted with seed of the variety Lincoln, the other two with seed of the variety Hawkeye. Corresponding plantings were made in K-infested flats. Of the 140 seeds planted per flat, 70 had been treated with Spergon at the rate of 2 oz. per bu., the other 70 being left untreated. As checks, exactly similar plantings of the two varieties were made in non-infested steamed soil.

The results were very definite. Not a single healthy seedling appeared in the eight flats of infested soil, whereas, in the check flats, the seed germinated to the extent of practically 100 per cent and gave rise to vigorous, healthy seedlings (Figure 4). The results would suggest the ineffectiveness of treatment of seed when seed is planted in heavily infested soil.

*Exploratory Tests Under Conditions of Partial Control of the Environment*

On August 13, Spergon-treated seeds of the variety Hawkeye were planted in steamed soil in 2-inch pots (two seeds per pot). On August 27, when the plants, then two weeks old, had reached a height of about 5 inches and were in the early, second-true-leaf stage, they were transferred with as little root disturbance as possible to 4-inch pots containing steamed soil. Prior to the transfer of plants, the soil in some of the pots had been infested with the E and in others with the K isolate of *Pythium*. In the rest of the pots the soil remained non-infested. Ten E- and ten K-infested pots were left in the greenhouse where, during the ensuing two weeks, the temperature ranged from 15.5° to 36.5° C., the average daily range for the period being from 18.5° to 31.5° C. Duplicate series of the E- and of the K-infested pots were transferred to a growth chamber where the temperature was maintained at 18.5° C., and where hours of light and darkness corresponding with those to which the plants in the greenhouse were exposed, were obtained by automatically-controlled, fluorescent lights. Plants growing in non-infested soil in the two environments served as checks.

Within three days of their being transferred to infested soil, plants in both environments showed definite indications of abnormality as evidenced by the yellowing and drooping of their cotyledons and the flaccidity of their unifoliate or first true leaves (Figure 1). Later, the flaccidity involved the whole plant and became especially pronounced in the plants growing in the growth chamber. When the soil in the pots in the chamber was not kept in an almost saturated condition, the plants showed a definite wilting and, when water was withheld temporarily from some of the E- and of the K-infested pots, the plants died. In corresponding pots treated similarly in the greenhouse, the plants wilted but did not die. From the symptoms



PLATE I

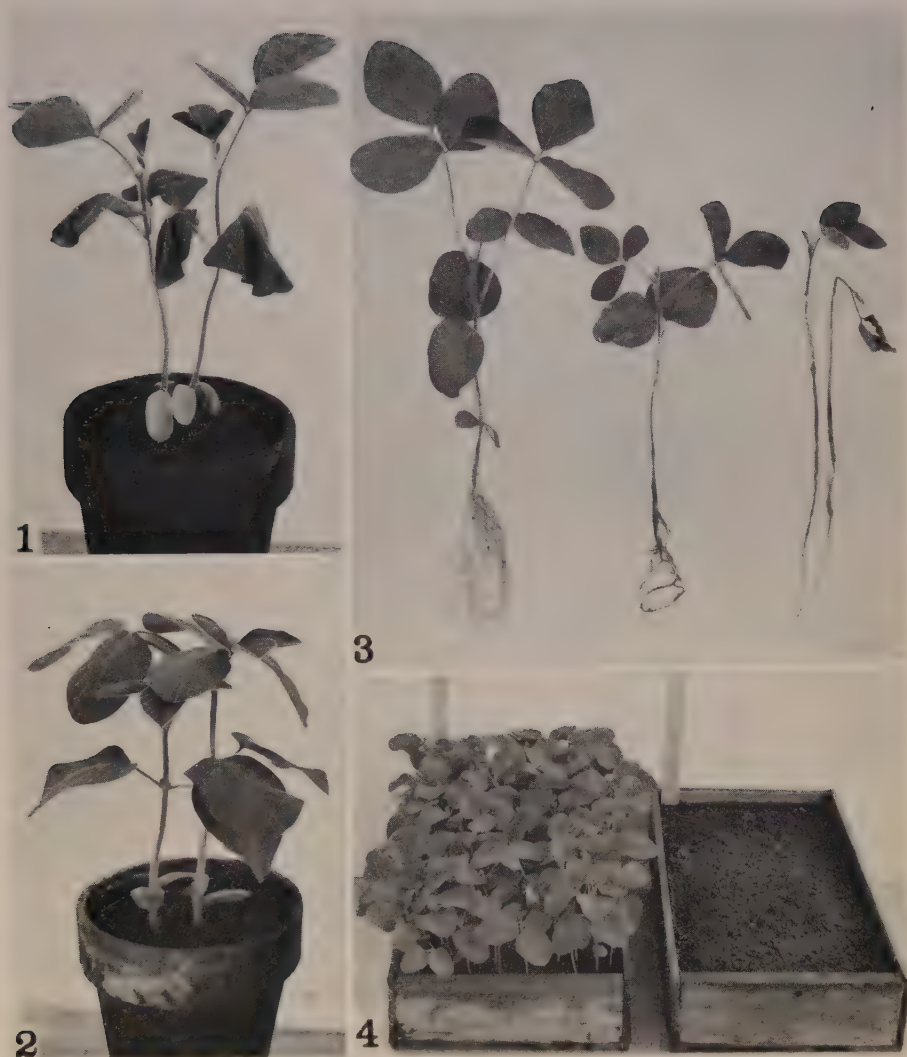


FIGURE 1. A 17-day-old Hawkeye plant showing early symptoms of *Pythium* stem and rootrot 3 days after being transplanted to artificially-infested soil. Note yellowed, drooping cotyledons and flaccid unifoliate or first true leaves. FIGURE 2. Corresponding check plant grown in non-infested, steamed soil. FIGURE 3. Transplants 15 days old, of the variety Hawkeye, grown for 8 days (*left*) in greenhouse in non-infested, steamed soil (*centre*) in greenhouse, and (*right*) in the 18.5° C. growth chamber, the two latter in artificially-infested steamed soil. FIGURE 4. (*Left*) Seedlings 10 days old, of the variety Lincoln, grown from Spergon-treated seed in non-infested, steam soil. (*Right*) Complete non-emergence of corresponding seed in artificially-infested, steamed soil.



FIGURE 5. Effect of temperature on Pythium stem and root rot of soybean as manifested by seeds (left in each figure) and transplants (right in each figure) grown for 17 days in artificially-infested, steamed soil (*Pythium*). Background of each figure shows growth of corresponding seeds and transplants in non-infested steamed soil.



FIGURE 6. Effect of temperature on Pythium stem and root rot of soybean as manifested by seeds (left in each figure) and transplants (right in each figure) grown for 17 days in artificially-infested, steamed soil (*Pythium*). Background of each figure shows growth of corresponding seeds and transplants in non-infested steamed soil.



displayed by the above-ground parts of the plants, it was quite apparent that the effects of the fungus were more severe in the cooler than in the warmer environment. The effect of the E isolate seemed to be quite similar to that of the K isolate. Check plants growing in both environments remained healthy, but those in the greenhouse were more robust than those in the growth chamber.

On September 4, eight days after their transfer from the 2-inch to the 4-inch pots, plants were removed from the infested and the non-infested soil and their roots examined. Those grown in infested soil in the 18.5° C. environment were most severely affected. As is shown in Figure 3, they had very little of their root system left and their above-ground parts were in a state of more or less complete collapse. Plants grown in the infested soil in the higher and more variable temperature of the greenhouse had been attacked somewhat less severely by the fungus. The root system of these plants was discoloured, necrotic, and very much depleted. Check plants remained robust and healthy and possessed an abundant root system. The fungus was readily reisolated from the brownish, water-soaked lesions on the rootlets of plants grown in the infested soil.

This experiment added confirmation to the fact that the two isolates of the fungus were highly pathogenic to the soybean and it indicated also that the pathogenic capabilities of the fungus isolates were modified by both the temperature and the moisture of the soil, the former being probably the more important of the two.

#### *Tests Conducted Under Conditions of Precise Control of Temperature*

On October 11, a quantity of steamed, composted soil was infested with the *Pythium* isolate E, by adding, per cubic foot of soil, 150 grams of a cornmeal-sand mixture on which the fungus had grown for ten days. Eight pans, each 16.5 × 9 × 4.5 in., were filled with 600 cu. in. of the infested soil. In each of four of the pans, 64 Spergon-treated seeds of the variety Lincoln were planted and, to each of the other four pans, ten 10-day-old plants of the same variety were transferred from 2-inch pots of steamed soil. One seed-containing and one plant-containing pan were transferred to each of four Wisconsin temperature tanks adjusted to 11-12°, 15-16°, 21-22°, and 26-27° C. For each tank, a corresponding pair of pans in which seed was planted and plants were set in non-infested, steamed soil, were prepared. Sufficient water was added then and subsequently throughout the duration of the test to maintain the soil in a moderately moist condition.

Almost all of the seed planted in the infested soil was completely destroyed. In the 26-27° C. tank, about six seedlings did "break ground" but, after 11 days, only two of these six approached normality (Figure 5). In the other three tanks, although the cotyledons of a few seeds did become visible, they were attacked almost immediately and destroyed. All seed planted in the non-infested soil germinated to the extent of practically 100 per cent and healthy seedlings developed at all four temperatures, although growth at 11-12° C. was much slower and less robust than at 26-27° C. (Figures 5 and 6).

At 26-27° C., little effect was noted on the transplants in the infested soil. In fact, as Figure 5 shows, they grew into almost as robust and healthy plants as their counterparts in the non-infested soil. At 11-12° C.,

the plants in the infested soil showed few signs of development. The cotyledons of these plants soon turned yellow and most of them dropped off prematurely. In addition, the plants wilted and remained in a more or less flaccid condition (Figure 6) throughout the duration of the experiment. At 15-16° C., the plants in the infested soil were less severely affected and still less so at 21-22° C. The evidence was clear that, the lower the temperature, the more severely were the plants attacked by the fungus. All transplants in the non-infested soil remained healthy regardless of temperature, although those grown at 11-12° C. showed less vigour of growth than those grown at 26-27° C.

#### IDENTIFICATION OF THE CAUSAL ORGANISM

The *Pythium* isolates obtained from diseased plants from the three different sources indicated in the introductory paragraph were studied as to their growth characteristics on several kinds of culture medium, including potato-dextrose agar, oatmeal agar, corn meal agar, and plain water agar. Morphologically, they all appeared to be alike. Above, it was shown that the E and the K isolate, obtained from plants from Essex and Kent counties, respectively, were identical as to their pathogenic capabilities. Thus, all the available evidence indicated that the various isolates were representatives of the same fungus. This fungus conforms more closely with *Pythium ultimum* Trow as defined by Middleton (13) than with any other species of the genus. *P. ultimum* has not heretofore been reported as a pathogen of the soybean. As recorded above in the literature review, the only other species of *Pythium* that has been mentioned as pathogenic to the soybean is *P. debaryanum*. Following is an excerpt from Middleton's (13) description of *P. debaryanum*: "Antheridia 1 to 6 per oogonium, monoclinalous and diclinalous, when monoclinalous arising some distance below the oogonium, not adjacent to it". In the present studies many hundreds of oogonia of the organism concerned were examined critically. Almost without exception, each oogonium had attached to it a single antheridium that was of monoclinalous origin. On a few rare occasions, oogonia were observed with two antheridia attached, one of the latter being of monoclinalous, the other of diclinalous origin. It is chiefly on the basis of its antheridial characteristics that the organism with which these studies are concerned was identified as *P. ultimum*\* rather than *P. debaryanum*.

In 1926, when Lehman and Wolf (8) named *P. debaryanum* as the causal agent of a rootrot of soybeans in North Carolina, they eliminated *P. ultimum* and a number of other species of *Pythium* on the basis that they were saprophytes. Since that time, *P. ultimum* has been reported as parasitic to many different plants. After re-reading Lehman and Wolf's description of the organism with which they worked, and examining their illustrations of the same, the present writers are of the opinion that, according to present conceptions, the *P. debaryanum* of Lehman and Wolf might possibly be more accurately identified as *P. ultimum*. If their organism had been *P. debaryanum*, they should have had no particular difficulty in getting the sporangia to germinate with the production of zoospores, yet

\* Since the above was written, confirmation of the writers' diagnosis was received from John T. Middleton, University of California Citrus Experiment Station, Riverside, Calif., to whom a culture of the isolate from soybean was submitted.



their numerous efforts to induce germination of these bodies resulted either in the conidial type of germination or no germination at all. The sporangia of *P. ultimum* much more commonly but not invariably (1) germinate by the production of germ tubes.

### DISCUSSION

At the moment, the chief interest that attaches to the discovery of *Pythium* stem and rootrot of soybean in Ontario is academic: a relatively rarely-occurring disease of this particular host has been found in a new geographic location. Last year (1951) the disease was of almost negligible importance economically. There is no assurance, however, that it will continue to be unimportant. Recently Weiss (18) has drawn attention to three presumably minor diseases of the soybean that suddenly flared into importance in different areas in the United States. The causal organism of the disease under consideration in the present paper is one of the most ubiquitous of the species of *Pythium* and is undoubtedly indigenous to the soil in Ontario. Each year soybeans are being grown more extensively and more intensively in the southwestern part of the province. Thus, conditions would seem to favour a possible rapid build-up and spread of the pathogen unless precautionary measures are adopted. It has been shown above that seed treatment offers no protection against the disease, over a wide range of temperature, if the soil is heavily infested. Of primary importance as a preventive measure would seem to be the adoption of a crop-rotation program in which soybeans should not follow soybeans, especially on land where any trace of the disease has appeared. Such a program would be all the more advisable for the control also of the now firmly-established stem canker disease, caused by *Diaporthe phaseolorum* var. *batatatis*, which is currently the most serious threat to the successful growing of soybeans in Ontario.

### SUMMARY

In early July, 1951, a stem and rootrot affecting a number of varieties of soybeans was found almost simultaneously in Essex and Kent counties in southwestern Ontario. Affected plants, occurring singly or in small groups, are dwarfed and show first a wilted, then a necrotic, brown, dried-out condition. The causal organism was identified as *Pythium ultimum* Trow, which heretofore has not been reported as pathogenic to the soybean. The disease is favoured by low soil temperature, although, in heavily-infested soils, the fungus is capable of destroying seed over a relatively wide range of soil temperatures.

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# CHEMICAL CONTROL OF WEEDS IN PEAS<sup>1</sup>

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The control of broad-leaved weeds in peas grown for processing presents an important problem. In the Annapolis Valley district of Nova Scotia the most common weed occurring in pea-fields is wild radish, *Raphanus raphanistrum*. In many instances the stand of this weed is so thick that it reduces yields to an unprofitable level and it is difficult to get farmers to grow this crop. The canners also have an interest in this matter since both vining time and hand-labour time to remove the weed seed pods are increased. The tests reported here were an attempt to discover a practical chemical weed-control procedure.

Successful chemical weeding of peas was reported by Westgate and Raynor (1) from California in 1933 using sprays of sodium dinitro-ortho cresylate. The use of sprays of ammonium dinitro-ortho-sec-butyl phenol has been reported by Barrows and Grigsby (2) from Michigan in 1945 and from Nova Scotia (3) in 1946. Roberts and Blackman (4) from England reported in 1950 the successful use of ammonium dinitro-sec-butyl phenol sprays for the control of annual weeds in leguminous crops. They report difficulty in killing *R. raphanistrum* when this weed is approaching the flowering stage.

More recently the successful use of chemicals applied to the soil prior to emergence of the peas has been reported from this Station and other centres at regional weed control conferences (5, 6 and 7).

## METHODS AND MATERIALS

The experiments were conducted at the Experimental Station, Kentville, N.S., in 1949, 1950 and 1951. Plots in 1949 and 1950 consisted of drill-width strips of sufficient length to make 1/100-acre plots. In 1951, to reduce the amount of labour required for harvesting, the length of the plots was reduced to make plots of 1/620 acre. The plots were replicated three times in randomized blocks. Seeding was done with a 13-run seed drill on land previously fertilized with 3-15-6, applied broadcast at 1000 pounds per acre and harrowed in. The land was rolled following seeding. In 1949 the variety Thomas Laxton was used and in 1950 and 1951, Perfection. After harvesting by mowing, the pods were removed from the vines by hand and shelled in a small mechanical sheller.

✓ The materials used are given in Table 1.

Due to differences in season, the seeding dates varied somewhat in different years. The earliest was May 14 in 1949, and the latest May 25 in 1951. Judging the effect of moisture and temperature conditions at the time of seeding, an attempt was made to apply pre-emergence treatments within three days of emergence of the pea seedlings.

Post-emergence treatments were applied when the peas were from 6 to 8 inches tall, and at this time wild radish plants were always showing a few blossoms.

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TABLE 1.—MATERIALS USED AS HERBICIDES

Treatment	Years tested	Method of application
<i>Pre-emergence treatments</i>		
Granular calcium cyanamid	3	Dry on the soil
Isopropyl ester 2,4-D	3	Spray
Low volatile ester 2,4-D (mixture of propylene glycol and butyl ether esters)	2	Spray
XP4OG (xanthogen disulfide 50% plus oils and emulsifier)	2	Spray
Amine salts DNOSB (alkalonamine salts of the ethanol and isopropanol series of dinitro-o-sec-butyl phenol 54%)	2	Spray
<i>Post-emergence treatments</i>		
Calcium cyanamid dust	2	Dust to previously wetted foliage
Sodium cyanamid	1	Spray
Potassium cyanate	1	Spray
Sodium isopropyl xanthate	1	Spray
Ammonium DNOSB (ammonium salt dinitro-o-sec-butyl phenol 13.7%)	3	Spray

*Note:* All materials applied as sprays were diluted with water to a volume of 35 gallons per acre and in addition ammonium DNOSB was used in dilutions equal to 70 gallons per acre. Sprays were applied with a specially designed sprayer (Figure 1).

In 1949 and 1950 actual counts of weed seedlings were made following the emergence of the peas. In 1951 only visual estimates were made.

### RESULTS

The results of estimates of weed population in the plots where pre-emergence treatments were used are given in Table 2.

TABLE 2.—COMPARISON OF HERBICIDES USED AS PRE-EMERGENCE TREATMENTS

Treatment	Number of weeds per square yard		
	1949	1950	1951*
Granular calcium cyanamid, 60 lb. per acre	333	—	—
Granular calcium cyanamid, 100 lb. per acre	—	93	150
Granular calcium cyanamid, 200 lb. per acre	129	108	150
Granular calcium cyanamid, 400 lb. per acre	—	108	150
Granular calcium cyanamid, 600 lb. per acre	15	—	—
Isopropyl ester 2,4-D, $\frac{3}{4}$ lb. acid per acre	144	39	75
Low volatile ester 2,4-D, $\frac{3}{4}$ lb. acid per acre	—	60	75
XP4OG, 3 qt. per acre	—	92	75
Amine salt DNOSB 54%, 4 qt. per acre	—	—	Nil
Amine salt DNOSB 54%, 8 qt. per acre	—	Nil	Nil
Untreated	651	318	800

\* Visual estimates only.

Harvesting was done when the peas had reached a stage of maturity suitable for canning.

Table 3 gives yields of shelled peas together with estimates of weed density at harvest time.



TABLE 3.—COMPARISON OF DIFFERENT HERBICIDES. YIELDS OF SHELLED PEAS AND DENSITY OF WEEDS

Treatment	Yield, in pounds per acre		Estimated density of weeds, control = 100	
	1949	1950	1949	1950
<i>Pre-emergence treatments</i>				
Granular calcium cyanamid, 60 lb. per acre	2660	—	100	—
Granular calcium cyanamid, 100 lb. per acre	—	838	—	75
Granular calcium cyanamid, 200 lb. per acre	3000	516	70	75
Granular calcium cyanamid, 400 lb. per acre	—	791	—	70
Granular calcium cyanamid, 600 lb. per acre	3820	—	25	—
Granular calcium cyanamid, 600 lb. per acre	3440	1266	70	50
Isopropyl ester of 2,4-D, $\frac{3}{4}$ lb. acid per acre	—	1018	—	50
Low volatile ester of 2,4-D, $\frac{3}{4}$ lb. acid per acre	—	978	—	—
XP4OG, 3 qt. per acre	—	—	—	—
Amine salt DNOSB 54%, 4 qt. per acre	—	1541	—	10
Amine salt DNOSB 54%, 8 qt. per acre	—	—	—	10
<i>Post-emergence treatments</i>				
Calcium cyanamid dust, 100 lb. per acre	2500	951	75	75
Calcium cyanamid dust, 200 lb. per acre	2400	—	75	—
Sodium cyanamid, 50 lb. per acre	3260	—	50	—
Potassium cyanate, 21 lb. per acre (3% solution)	—	—	—	80
Sodium isopropyl xanthate, 15 lb. per acre	—	677*	—	10*
Ammonium DNOSB, 13.7%, $1\frac{1}{2}$ qt. per acre (in 70 gal. water)	2620	1085	50	25
Ammonium DNOSB, 13.7%, 3 qt. per acre (in 70 gal. water)	2020	1064	40	25
Ammonium DNOSB, 13.7%, $1\frac{1}{2}$ qt. per acre	—	1695	—	10
Ammonium DNOSB, 13.7%, 3 qt. per acre	—	1541	—	10
Untreated	2500	563	100	100
"t" at 5% point $\times$ SE difference between treatment means	930	454	—	—

\* Such severe injury to peas that this material was not considered worth further trial.



FIGURE 1. Small plot sprayer consisting of compressed air reservoir and bottle for spray solution, mounted on bicycle wheels.

Table 3 shows that the yield of shelled peas may be considerably influenced by herbicide treatment. This was particularly noticeable in 1950, a very dry year, when competition for moisture must have been a critical factor. In 1949 weed control with some of the treatments was good, but did not result in significantly increased yields. In 1951 yields were generally low, and this is partially accounted for by poor seed germination and resulting spotty stand. Weed control in 1951 was generally poor except on the plots where amine salt of DNOSB was used as a pre-emergence treatment.

There did not seem to be any advantage in applying the ammonium salt of DNOSB in high volume as a post-emergence treatment. In fact in 1950, weed control was somewhat better at the 35-gallon per acre rate than at the 70-gallon rate. In 1951 this material gave generally poor results at both rates and volumes of application.

The appearance of some of the plots in 1951 at the time when wild radish was in full bloom is illustrated in Figures 2 to 5.

Poor weed control in 1951 was no doubt due to cool, damp weather during the growing season. This reduced the effectiveness of the treatments, at the same time stimulating weed growth. Even under these conditions the amine salt of DNOSB gave good protection right up to harvest time, as it did in 1950, an extremely dry year.

#### DISCUSSION AND CONCLUSIONS

The results presented here have shown that both pre-emergence and post-emergence treatments with herbicides will control wild radish in peas.





FIGURE 2. Amine salt DNOSB 54%, 8 qt. per acre, pre-emergence, 1951.



FIGURE 3. Amine salt DNOSB 54%, 4 qt. per acre, pre-emergence, 1951. Untreated plot in background.





FIGURE 4. Ammonium DNOSB 17%, 3 qt. in 70 gal. water per acre, post-emergence, 1951.



FIGURE 5. Granular cyanamid, 400 lb. per acre, pre-emergence, 1951. Amine salt DNOSB, 54% pre-emergence plot in background.



For post-emergence treatments it is necessary to use herbicides that kill by contact, selective action being due to differential wetting of the foliage. Success depends to a great extent on the weather at the time of and immediately following application. In 1949 and 1951, when treatments were followed by cool damp weather, post-emergence sprays were not particularly effective in controlling wild radish when this plant was approaching bloom stage. Sodium cyanamid may be an exception in this respect but needs further trial. In 1950, a year when hot, dry weather followed treatment, ammonium DNOSB gave excellent control, but calcium cyanamid dust and potassium cyanate sprays were not effective. Sodium isopropyl xanthate, used in one year only, caused such severe injury to the peas it was not considered worth a further trial.

For pre-emergence treatments it is necessary to have a herbicide that will kill germinating weed seedlings but will not affect the germinating peas if applied a few days before they emerge. Secondly, the effect of the treatment should be as long-lasting as possible.

In these tests materials used as pre-emergence treatments may be divided into two classes:

(a) Materials which suppress weed growth for a short period after application. They may be of limited value when weed populations are small, but are of no practical use where weed populations are large. Materials in this class are compounds of 2,4-D, both volatile and low volatile esters, granular calcium cyanamid and xanthogen disulphide.

(b) Materials which suppress weed growth right up to harvest time. In these tests only one material, Alkalonamine salts of DNOSB, 54 per cent, was in this class. In both years tested, it gave practical freedom from weeds right up to harvest time. Used as a pre-emergence spray it offers an effective means of controlling weeds in peas grown for canning.

#### ACKNOWLEDGMENT

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# THE TOXICITY OF ALDRIN FOR GROWING TURKEYS<sup>1</sup>

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## REVIEW OF LITERATURE

During 1950 and 1951 the insecticide aldrin (compound 118) came into widespread use for the control of grasshoppers on the Canadian Prairies. No work appears to have been reported in the literature on the effect of aldrin when included in the diets of domesticated birds. Numerous requests from poultry raisers as to the effect on poultry of this insecticide prompted this study.

In studies on sub-acute (90 day) oral toxicity in rats, Borgman and co-workers (1) found that no mortality occurred in groups receiving as high as 75.0 p.p.m. of aldrin in the diet, but 8 out of 12 rats died within 90 days on a 150.0 p.p.m. level. It was estimated that 75 p.p.m. represented a daily dose of about 3.75 mg./kgm. body weight. They also showed that rabbits are more susceptible to continued oral dosages of aldrin than rats. A rate of 2.5 mg./kgm. body weight per day gave mortality in 90 days, but no mortality occurred at 1.25 mg./kgm.

In tissue distribution studies of aldrin fed to rats, these workers found that, in acute dosages, biological assays revealed largest concentrations in the kidney and liver, with less in the heart, lungs and blood. Tissue analyses after sub-acute administration of aldrin at 50 and 75 p.p.m. from 2 to 35 weeks revealed largest concentrations in liver and kidney tissue. Levels of 1 p.p.m. to 7 p.p.m. were found. Aldrin when fed to rats at levels ranging from 5.0 to 0.5 p.p.m. over periods of 14 to 32.5 weeks was found in the perirenal and omental fat in inconsistent amounts ranging from 0.25 to 12.0 p.p.m.

In pathological studies of rats and rabbits fed aldrin in amounts which proved lethal after a number of weeks' dosage, Dutra (3) found that lung damage, as evidenced by extensive pulmonary edema and congestion, was typical. There were also degenerative changes in liver, kidney and brain. In similar studies on large animals, Dutra (4) found that degenerative lesions of the liver, brain and kidneys resulted from doses of aldrin of 2 mgm./kgm. body weight, when administered for a number of days.

Toxicological studies on large animals by Kitselman *et al.* (5) revealed that, when sheep and cattle were fed alfalfa hay which had been treated with aldrin at the rate of 0.5 pounds per acre and cut 8 days after application, no toxic effects were exhibited during feeding periods of 169 and 213 days, respectively. Weight gains were normal. In tissues of cattle and sheep which died after being fed aldrin at daily levels of 2.0 mgm to 7.5 mgm./kgm. body weight, the chemical was recovered in amounts ranging from 50 p.p.m. to less than 1 p.p.m. Body and perirenal fat contained the highest amounts.

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For the purpose of determining the minimum toxic and maximum tolerable levels of aldrin in the diets of growing turkeys a series of experiments were set up during the summer of 1950.

### MATERIAL AND METHODS

Broad Breasted Bronze turkeys which were 8-10 weeks of age were divided, on the basis of weight, into equal sized groups for each experiment. Sexes were distributed equally on the basis of vent sexing. The number of birds per group was 10 in Experiments 1 and 2 and 20 in Experiment 3. They were confined to equal sized floor pens with clean wood shavings as litter. Food and water were supplied ad libitum. Body weights and feed consumption data were recorded weekly. Twice daily observations of the birds' condition were made.

The basal rations shown in Table 1 were used to make up diets containing varying levels of technical aldrin.

The aldrin used was a commercial preparation containing 2.5 pounds of technical aldrin per imperial gallon. An imperial gallon weighed ten pounds. The procedure used in Experiments 1 and 2 was to prepare a diet containing 1000 p.p.m. of aldrin and then to dilute this by thorough mixing with the required amount of basal to produce diets containing the required levels. The required amount of aldrin was diluted with 1000 ml. of water and was applied with a hand sprayer. The feed was placed in a motor-driven 2 cu.ft.-cement mixer and the emulsion sprayed into it as the mixer rotated. Ten minutes were required to apply the 1000 ml. of emulsion. In Experiment 3 the practice of mixing a stock diet was dropped in favour of separate sprayings for each level of aldrin. It was felt that this would result in a more uniform distribution of the insecticide.

### RESULTS AND DISCUSSION

#### *Experiment 1*

Diets containing 125, 250, 500 and 1000 p.p.m. of technical aldrin were fed in the first experiment. Those diets containing 250 p.p.m. or more proved unpalatable from the start. The diet containing 125 p.p.m. was eaten quite readily for the first few hours.

Within 12 hours birds on the diet containing 1000 p.p.m. were listless and by the time 36 hours had elapsed all birds on the treated diets were similarly affected. Very little feed was consumed after first toxicity symptoms appeared. They lost weight rapidly and died within a matter of days. Those birds on the control diets continued to make normal gains. Average survival time for the birds on treated diets was 4.2 to 5.8 days.

#### *Experiment 2*

In this experiment levels of 12.5, 25, 50 and 100 p.p.m. of technical aldrin were fed. All treated diets were eaten quite readily at the start but those birds receiving 25 p.p.m. or more in the diet lost their appetites within a few hours and showed symptoms of poisoning within 36 hours. Birds in these three groups lost weight rapidly and were quite emaciated prior to death. All birds receiving 25 p.p.m. or more succumbed within 10 days. Average survival time for these groups was 6.4 to 9.4 days.

TABLE 1.—BASAL RATIONS

Ingredients	Weights	
	Diet A	Diet B
	lb.	lb.
Ground barley	20.00	17.00
Ground oats	10.00	7.00
Ground wheat	41.64	32.80
Whole wheat	—	11.00
Whole oats	—	7.00
Vita grass	5.00	5.00
Meat meal (60 per cent)	21.00	17.00
Bone meal	—	0.90
Ground limestone	1.36	1.30
Salt	0.50	0.50
Fish oil (1200A-200D)	0.50	0.50
Manganese sulphate	9.30 gm.	9.30 gm.
Potassium iodide	0.23 gm.	0.23 gm.
Total	100.00	100.00

Birds on the 12.5 p.p.m. diet were not affected so severely. Both sexes on this level gained weight slightly during the first seven days. First death occurred on the seventh day but all succumbed within 25 days.

### *Experiment 3*

In this experiment one hundred and twenty 76-day old turkeys were divided into six equal groups. Diets containing 1.5, 3.0, 6.25, 12.5 and 25 p.p.m. of technical aldrin were fed. The basal diet used during the first two weeks of the experiment is shown as "A" in Table 1. That used during the last four weeks is shown as "B" in Table 1.

Table 2 shows the results of the feeding of the low levels of aldrin.

Diet 29G resulted in 100 per cent mortality in 14 days. Diet 29F resulted in the death of only three birds, on the 12th, 16th and 34th day, respectively. All birds on this diet exhibited mild symptoms of toxicity. They were quite listless throughout the test period and were easily excited and struggled considerably when handled. Cold shanks was a common observation throughout the 6-week period. The effect on body weight of the various diets is shown in Table 2. All diets in which aldrin was included caused a depression in weight gains below that of the controls. These differences were shown to be statistically significant for both males and females for diets 29L and 29F. In the case of diet 29K the difference was significant only in the case of males. The apparent non-toxic level of aldrin in the diet of growing turkeys of this age and under the conditions of this experiment is therefore between 1.5 p.p.m. and 3.0 p.p.m.

All surviving birds from Experiment 3 were placed on an aldrin free diet on range immediately following the six-week period on treated diets. Four days after being moved, very cold weather set in. All but one of the survivors from diet 29F (12.5 p.p.m. aldrin) succumbed during the first five days of this low-temperature period. No other groups suffered any mortality. This would indicate that the systemic poisoning resulting from birds being fed diet 29F was severe enough to cause them to be unable to meet the ordinary stresses which a normal bird could withstand quite successfully.

TABLE 2.—THE EFFECT OF VARYING LEVELS OF ALDRIN ON BODY WEIGHT, FEED CONSUMPTION AND MORTALITY OF GROWING TURKEYS

Diet number	Technical aldrin content	Sex and number	Average initial weight	Average weight end of 6 weeks	Mortality end of 6 weeks	Total feed consumption
29A	p.p.m. None	9 M	lb. 4.8	lb. 11.5	—	lb. 414
		11 F	4.1	8.8	—	
29J	1.50	8 M	4.8	10.9	—	394
		12 F	4.1	8.5	—	
29K	3.00	10 M	4.8	10.9	—	390
		10 F	4.0	8.4	—	
29L	6.25	10 M	4.7	10.2	—	391
		10 F	4.1	8.1	—	
29F	12.50	9 M	4.8	9.7	1	330
		11 F	4.1	7.8	2	
29G	25.00	10 M	4.7	—	10*	30
		10 F	4.1	—	10*	

\*Average survival time 8.2 days



It has been demonstrated by Decker and co-workers (2) that when aldrin was applied as a spray to alfalfa, sweet clover and red clover at the rate of 0.25 pounds per acre, the average initial deposits on these crops were 13 p.p.m., 31 p.p.m. and 29 p.p.m., respectively. The presently recommended rate of application for grasshopper control on the prairies is 0.125 pounds per acre. It is apparent from a study of Table 2 that the above concentrations are in line with those required to produce toxicity symptoms over a six-week period. Decker *et al.* (2) have shown that at the end of two days just under 50 per cent of the original deposit remained on clover foliage, with about 35 per cent left at four days, 15 per cent at seven days, and complete disappearance in about 21 days. The possibility of growing turkeys ingesting harmful quantities of aldrin if allowed to forage over freshly sprayed pastures requires to be further investigated. It should also be considered that the carotenoid layer on the feet of poultry offers no barrier to the insecticide chlordane\* and this may hold true for aldrin as well.

### *Toxicity Symptoms*

Toxicity symptoms as recorded in Experiments 1 and 2 were characterized by intermittent periods of listlessness and excitement. These were observed in the early stages of poisoning, but the periods of excitement became more lengthy within a matter of hours. Muscular incoördination followed and rapidly became so severe that the birds could not remain standing. Considerable squawking, particularly when approached by other birds, was noted. The shanks of the affected birds were cold to the touch. Kicking, fluttering and gasping for breath, with the head drawn up over the back were common in the final stages of semi-coma. Death generally followed in a few minutes.

### *Post-Mortem Findings*

Post-mortem examinations did not reveal any clear-cut findings. Rigor mortis set in very rapidly. Some congestion of the liver and kidneys was noted, with considerable congestion of blood and some edema and haemorrhage in the lungs. No irritation could be found in the digestive tract, but in all cases the crop was somewhat distended with gas.

Early in October, 1950 a freshly sprayed sample of feed containing 25 p.p.m. of aldrin, a sample of the basal diet and eight growing turkeys 10 weeks of age were sent to the Department of Veterinary Science at the University of Saskatchewan. The resulting report\*\* from that institution revealed toxicity symptoms in agreement with those reported above. It was stated that the symptoms observed were fairly typical of a derangement of the central nervous system, with some indication of an interference with heart action and the general circulation during the later stages.

It was also indicated in the report that the autopsies held on the dead birds failed to reveal any well marked pathological changes. Congestion of the entire venous system, the lungs, liver and kidneys was noted. The intestine showed evidence of congestion, the mucous membrane being dark red in colour and coated with a thick mucous exudate. This, it was

\* Lehman, A. J., Chief Division of Pharmacology, Food and Drug Administration, Washington, D.C. *Personal communication.*

\*\* Millar, J. L., Professor of Veterinary Science, University of Saskatchewan, Saskatoon, Sask. *Personal communication.*

pointed out, appeared to be associated with an interference with venous circulation. The brain and other parts of the nervous system appeared to be normal. From these findings it was concluded in the report that aldrin, when ingested at a level of 25 p.p.m. in the feed of growing turkeys, does not have an irritating effect on the digestive system and its chief action appears to be confined to an interference with the function of the brain and the nervous system.

### SUMMARY AND CONCLUSIONS

In three experiments aldrin was added to the diets of growing turkeys at 1.5 p.p.m. to 1000 p.p.m., technical aldrin. Levels of 100 p.p.m. or more in the diet caused the feed to be quite unpalatable. All levels above 25 p.p.m. were highly toxic. Average survival time was 4.2 days for 1000 p.p.m. and 8.2 days for 25 p.p.m. A level of 12.5 p.p.m. caused mortality of 3 birds out of 20 in a 42-day feeding period.

All technical aldrin levels above 3.0 p.p.m., caused a significant depression in growth rate for both males and females. A level of 3.0 p.p.m. caused a significant growth depression for males only. It was shown that birds which had previously received 12.5 p.p.m. of technical aldrin in the diet for a 42-day period suffered extremely high mortality when returned to an aldrin-free diet on range, under adverse weather conditions. The control birds and those which had received lower levels of aldrin suffered no mortality under similar conditions.

Toxicity symptoms and post-mortem findings are outlined.

### ACKNOWLEDGMENTS

The authors wish to thank J. L. Miller, Professor of Veterinary Science, University of Saskatchewan, Saskatoon, Sask., for his co-operation in checking toxicity symptoms and post-mortem findings.

### ADDENDUM

Subsequent to the completion of this paper the authors secured an abstract\* of the results of a similar experiment with 3- and 6-week-old chickens which was reported at the Annual Meeting of the American Association of Economic Entomologists, Denver, Colorado. The lowest level of aldrin fed was 25 p.p.m. All chickens of both age groups died on this level. It was concluded that the LD-50 for single acute doses lies between 10 and 15 mgm./kgm. for 6-week-old chickens.

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# PRELIMINARY INVESTIGATIONS IN CHEMICAL CONTROL OF THE PALE WESTERN CUTWORM, *AGROTIS ORTHOGONIA* MORR. (LEPIDOPTERA: PHALAENIDAE)<sup>1</sup>

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## INTRODUCTION

Cultural methods for the control of the pale western cutworm, *Agrotis orthogonia* Morr., have been recommended and widely used in Western Canada for many years. These are described by Seamans (6), but none of the recommended practices is effective in reducing infestations after damage becomes evident. The larva feeds almost entirely below ground and, therefore, is not controlled by poisoned baits. In this respect it differs from climbing and surface-feeding cutworms. In previous investigations, chemicals applied to the soil or to the seed were not effective. Materials previously tested are described in an unpublished manuscript by Seamans (5).

The recent introduction and successful use of highly toxic chemicals in controlling soil-infesting insects led to investigations of effects on the pale western cutworm. Laboratory tests of the contact toxicity of 15 compounds to the larvae, made by Brown *et al.* (2) at the request of the senior author, showed that the pyrethrins, DNOC, BHC, and chlordane were the most toxic. A group of these, including BHC and chlordane, was selected for field testing in west-central Saskatchewan in 1950 and 1951. The selection of insecticides and rates of application were based on availability, cost, and effectiveness on other insect pests.

## METHODS

In 1950, ten one-half-acre plots were laid out contiguously in a uniformly damaged portion of an infested field of wheat near Rosetown, Saskatchewan. Larval population counts were made before spraying and again 5 and 14 days after spraying in the treated plots and in an adjacent untreated area. Fifty square-foot soil samples from predetermined positions in each plot were examined for larvae. The post-treatment samples were taken at positions adjacent to those for the pre-treatment samples. As a reduction in population from causes other than insecticidal action occurred in the untreated sample, the percentage reductions in the treated plots were adjusted using Abbott's formula (1).

In the 1951 experiments the plots were placed in an infested wheat field near Pym, Saskatchewan, a few miles from the previous year's plots and on the same type of heavy clay soil. The plots were one-quarter of an acre in size, and check plots were alternated with treated plots. Population

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counts in the treated and the untreated plots were made 14 days after treatment. These counts also were based on the examination of 50 square-foot samples per plot.

Replication of treatments was not possible in either year because of the limited area of uniformly severe infestation, the difficulty of operating field-sized spray equipment on plots smaller than one-quarter of an acre, and the time-consuming method of assessment.

The five insecticides tested, each at two rates, were applied as water emulsions directly to the soil surface and vegetation by a low-pressure, low-volume boom sprayer delivering four gallons of spray per acre. Sprays were applied on June 15 in 1950 and on June 13 in 1951.

Larvae were chiefly in the fifth and sixth instars at the time of spraying and were still causing damage. The average population before spraying was 80 larvae per 50 square-foot samples.

In both years the living larvae collected from each sprayed plot at the time of the first post-treatment assessment, 5 and 14 days after spraying in 1950 and 1951, respectively, were placed in individual salve tins and reared in the laboratory to obtain some measure of the residual action of each insecticide. A duplicate number of larvae were collected at the same time from an unsprayed portion of the field. Records were kept of larval mortality, parasitism, and pupation. Percentage mortalities of larvae collected from the treated and untreated plots were computed using Abbott's formula (1).

## RESULTS

The insecticides used, rates of application, percentage reductions in the field, and mortalities of reared larvae collected from the sprayed plots are shown in Table 1.

Table 1 shows a wide range of effectiveness between insecticides and rates of application, and also some inconsistency in results of comparable treatments in the two years' trials.

Dieldrin and chlordane were the most effective, each reducing the population by more than 80 per cent at the most effective rates applied.

Toxaphene and aldrin were next in effectiveness. Toxaphene did not give as great reduction in population in any of the trials as did aldrin at 8 ounces in 1950, but it was more consistent in its effectiveness. Aldrin, which gave the second highest mortality in 1950, gave virtually none in 1951. BHC (crude, 9 per cent gamma isomer) was comparable in effectiveness to toxaphene in 1950, but at the rates used it is relatively expensive and was replaced in 1951 by heptachlor. This insecticide proved superior to aldrin at the same rates of application but inferior to dieldrin, chlordane, or toxaphene at the most effective rates of application of these materials.

All of the insecticides have a substantial residual effect on pale western cutworm larvae. This is shown in the table by the percentage mortalities of larvae collected from sprayed plots one to two weeks after treatment and reared in the laboratory. Dieldrin was the most effective in this respect.

Pale western cutworm larvae are seldom observed on the ground surface. Twenty-four hours after spraying, hundreds of dying and dead larvae were found on the surface of the sprayed plots, the number increasing

with the toxicity of the insecticide. They had collected in depressions, footprints, and wheel-marks. Flocks of birds, particularly gulls and black-birds, appeared and fed on the surfaced larvae. The dead larvae disappeared after four or five days.

### DISCUSSION

Among cutworms there is considerable variation in susceptibility to various insecticides. This has been noted by Brown *et al.* (2) and by various workers. After tests with the variegated cutworm, *Peridroma margaritosa* (Haw.), in Wisconsin, Whipp and Chapman (8) suggest that control measures cannot be given for cutworms in general and that the relative toxicity of insecticides should be reported for each species involved. Similarly, Hill and Muma (4) note that differences in ecology, physiology, and habits of several species make generalization difficult on the control of cutworms with organic chemicals.

Differences are often found in the effectiveness of an insecticide applied at the same rate in the laboratory, in the greenhouse, and under various field conditions. Table 1 indicates that chlordane, dieldrin, and aldrin were less effective in 1951 than in 1950 at the same rates of application, whereas toxaphene was more effective in 1951. Gaines and Misticic (3) found that the application of one-half inch of simulated rain in laboratory tests reduced the toxicity of aldrin more than it did that of toxaphene and dieldrin. Similarly, they found that higher dosages were required in the field than in the laboratory or greenhouse and attributed this to a wider

TABLE 1.—EFFECTIVENESS OF VARIOUS SPRAYS AGAINST LARVAE OF THE PALE WESTERN CUTWORM, *Agrotis orthogonia* MORR., IN 1950 AND 1951 IN FIELD TESTS AT ROSETOWN, SASK.

Material	Rate per acre	Percentage reduction in plots 14 days after spraying		Percentage mortality of larvae collected in sprayed plots*	
		1950	1951	1950	1951
Chlordane	8	57	—	20	—
	16	63	42	14	54
	24	—	82	—	100
Dieldrin	4	81	26	64	42
	6	—	72	—	53
	8	81	—	42	—
Aldrin	4	35	14	25	24
	8	70	6	20	40
BHC, gamma isomer	2	48	—	22	—
	4	26	—	20	—
Toxaphene	16	34	—	8	—
	32	33	58	20	43
Heptachlor	4	—	12	—	58
	8	—	42	—	60

\* Larvae collected 5 and 14 days after spraying in 1950 and 1951, respectively.



range in relative humidity, sunshine, dew, or a combination of these factors in the field. The foregoing may explain the lower effectiveness of aldrin in 1951, when the weather was cool and showery, as compared with the warm and dry weather in 1950.

Dieldrin was the most toxic chemical tested in these experiments, 6 ounces of active ingredient per acre giving almost as satisfactory results as 24 ounces of chlordane. Weinman and Decker (7) found dieldrin more toxic than aldrin, lindane, chlordane, or toxaphene to the armyworm, *Pseudaletia unipuncta* (Haw.) [*Cirphis unipuncta* (Haw.)], either as a contact or as a stomach poison. At the present time dieldrin is not licensed for use in Canada but would be competitive in price and effectiveness with chlordane and toxaphene. Chlordane at 24 ounces gave satisfactory field control in these experiments. At 1951 prices its use at this rate appears practical for the protection of cereal crops in Western Canada. Toxaphene at 32 ounces is about similar in cost to 24 ounces of chlordane, but is not as effective. Brown *et al.* (2) also found toxaphene much less toxic than chlordane to *A. orthogonia* when these materials were compared for direct contact toxicity.

Corroborative evidence of the efficiency of chlordane and, to a lesser extent, of toxaphene, was obtained in tests in which these materials were applied as sprays in 20- and 40-acre infested fields in the Rosetown area. Chlordane at 16 ounces and toxaphene at 24 ounces per acre reduced populations to the point where no further damage occurred. In one instance, no further damage occurred in a field reseeded immediately after spraying with chlordane.

The successful use of insecticides for the control of the pale western cutworm in cereal crops depends upon the availability of materials that are not too expensive and upon the prompt action of the farmer at the first evidence of crop thinning by the cutworm. It is not generally feasible to determine before damage is evident whether or not cutworms are present in damaging numbers. Insecticides may also be of value in reducing populations on completely destroyed fields, making them safe for immediate reseeding. Otherwise a delay of several weeks to allow larvae to complete their development is usually necessary before a field can be safely reseeded.

The preliminary investigations reported herein show, for the first time, that field infestations of the pale western cutworm can be controlled with insecticidal sprays. Sprays were used rather than dusts since many farm operators possess weed sprayers that could be used with little or no modification. Further investigations are necessary to determine the rates of various insecticides for maximum control and to test new materials as they become available. Dusts, as well as sprays, should be included in further studies.

#### SUMMARY

Aldrin, BHC, chlordane, dieldrin, heptachlor, and toxaphene were applied as sprays to the soil surface to test their effectiveness as a control of the pale western cutworm, *Agrotis orthogonia* Morr. These materials were applied at various rates in 1950 and 1951 in west-central Saskatchewan. Chlordane, dieldrin, and toxaphene were the most effective.

## ACKNOWLEDGMENTS

The assistance of other laboratory personnel at Lethbridge and Saskatoon in making sample assessments, operating spray equipment, and conducting observations is acknowledged.

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# GERMINATION OF TREATED AND UNTREATED SEED OF WHEAT, OATS AND BARLEY OF DIFFERENT COMMERCIAL GRADES<sup>1</sup>

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## INTRODUCTION

Farmers in Canada, as a rule, sow cereal seed of good or reasonably good quality. There are times, however, when the only seed they have available has been exposed to adverse pre-harvest and harvest weather. Such exposure sometimes results in bleaching, moulding, and perhaps some sprouting of the seed. In years such as 1950, much of the seed grain may be injured by frost.

Treatment of cereal seed to destroy contaminants and to afford protection against soil micro-organisms has long been recognized to be a sound farm practice. There seems to be an impression, however, that seed damaged by adverse weather is improved by treatment to a greater degree than seed not so affected, although there appears to be scarcely any experimental evidence to support this view. Therefore, to obtain some definite information on the subject, the writers compared the germination of untreated seed and seed treated with Ceresan M (7.7 per cent ethyl mercury

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TABLE 1.—EFFECT OF TREATMENT ON GERMINATION OF WHEAT, OAT,  
AND BARLEY SEED OF DIFFERENT GRADES

Crop	Grade	Percentage increase in normal germination			Percentage of samples showing increase of 5 per cent or more
		Average	Highest	Lowest	
Wheat	1 Northern	12.6	93.4	— 8.5	78
	2 Northern	11.3	15.8	— 4.5	78
	3 Northern	7.7	35.2	— 4.0	70
	4 Northern	8.3	52.8	—15.1	68
	5 Wheat	9.3	68.6	—21.0	74
	6 Wheat	6.0	90.0	—39.2	72
Oats	2 C.W.	4.0	24.0	—17.7	44
	Extra 3 C.W.	6.4	43.5	— 9.7	58
	3 C.W.	7.7	84.2	—11.3	52
	Extra 1 Feed	8.6	56.2	—37.8	74
	1 Feed	9.2	86.3	—14.6	78
	2 Feed	10.2	177.0	—43.2	72
	3 Feed	9.8	100.0	—32.1	74
Barley	2 C.W. 6-row	5.4	26.6	—10.0	50
	3 C.W. 6-row	8.9	35.0	— 3.7	70
	4 C.W. 6-row	10.0	85.5	— 4.7	66
	1 Feed	6.0	107.0	—31.6	54
	2 Feed	6.8	56.6	—28.4	60
	3 Feed	3.8	42.8	—47.9	62



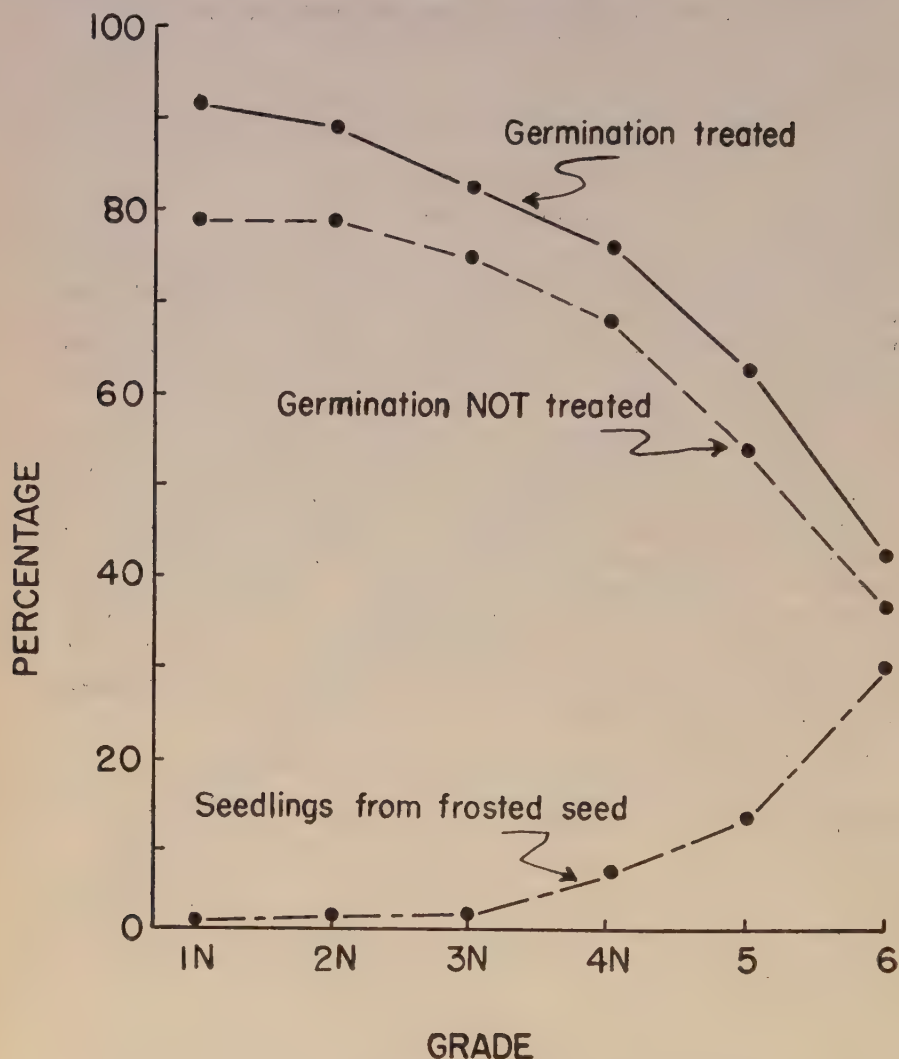


FIGURE 1. Germination (normal seedlings) of untreated and treated seed and the percentage of seedlings from seed injured by frost in different grades of wheat.

p-toluene sulfonanilide) of 50 samples each of six statutory or commercial grades of wheat, seven grades of oats, and six grades of barley. These samples were of the 1950 crop, and were obtained from the Grain Inspection Office (Western Division), Board of Grain Commissioners for Canada. They represented a range of market quality for each crop.

The samples were first freed from foreign material, and then 100-seed sub-samples were sown, treated and untreated, in a bed of unsterilized soil in a greenhouse. Ten days after seeding the seedlings were classified and counted, a record being kept of the percentage of normal plants, weak or deformed plants, lesioned plants, and plants showing malformation resulting from frost injury to the seed. The data obtained are summarized in the following section of this paper.

## EXPERIMENTAL RESULTS

It was observed early in the investigation that the germination of seed from samples of the same grade was far from uniform. Although, on the average, in all the three crops studied, germination declined as the grade declined, regardless of whether total germination or normal germination was taken into account, yet, in some samples of the lowest grade, the seed germinated as well as in the best samples of the highest grade. Where low germination occurred, the seed was injured mainly by frost, an injury which was found in all the grades considered but which was more severe in the lower grades than in the higher ones.

On the average, treatment of the seed increased germination by several per cent in all grades. The increase in wheat tended to be greater in the higher grades than in the lower ones (Figure 1), although the proportion of samples showing an increase of 5 per cent or more was about the same for all grades (Table 1). In oats, the average increase in germination, as

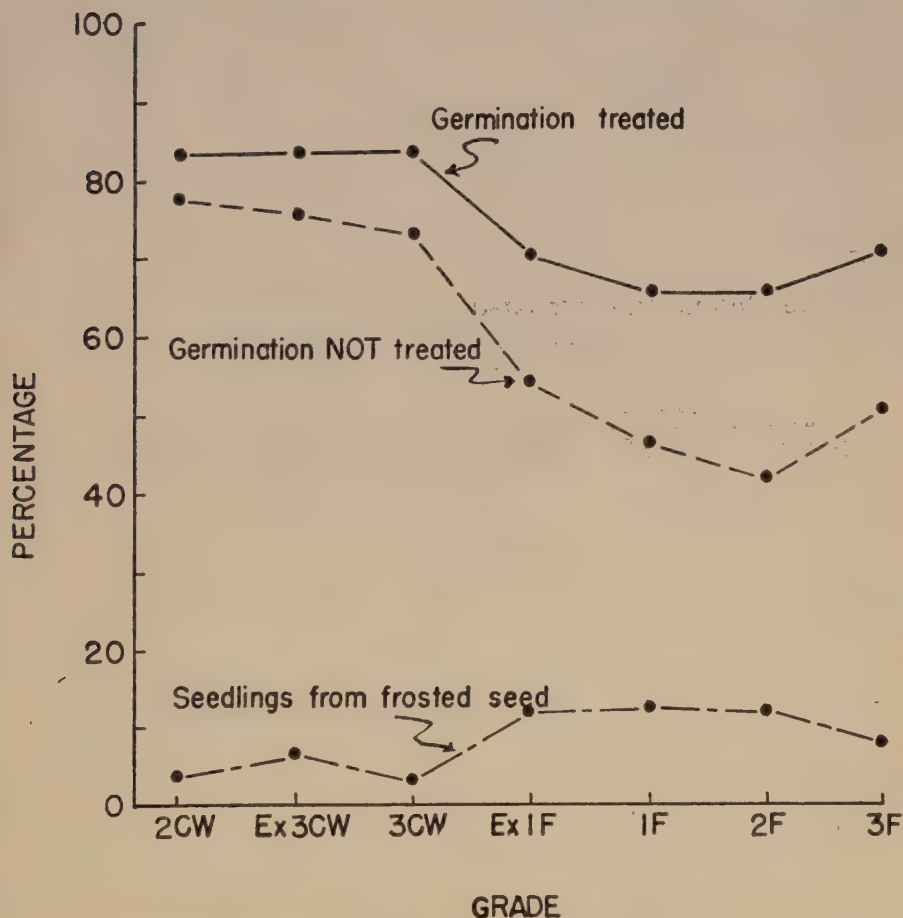


FIGURE 2. Germination (normal seedlings) of untreated and treated seed and the percentage of seedlings from seed injured by frost in different grades of oats.

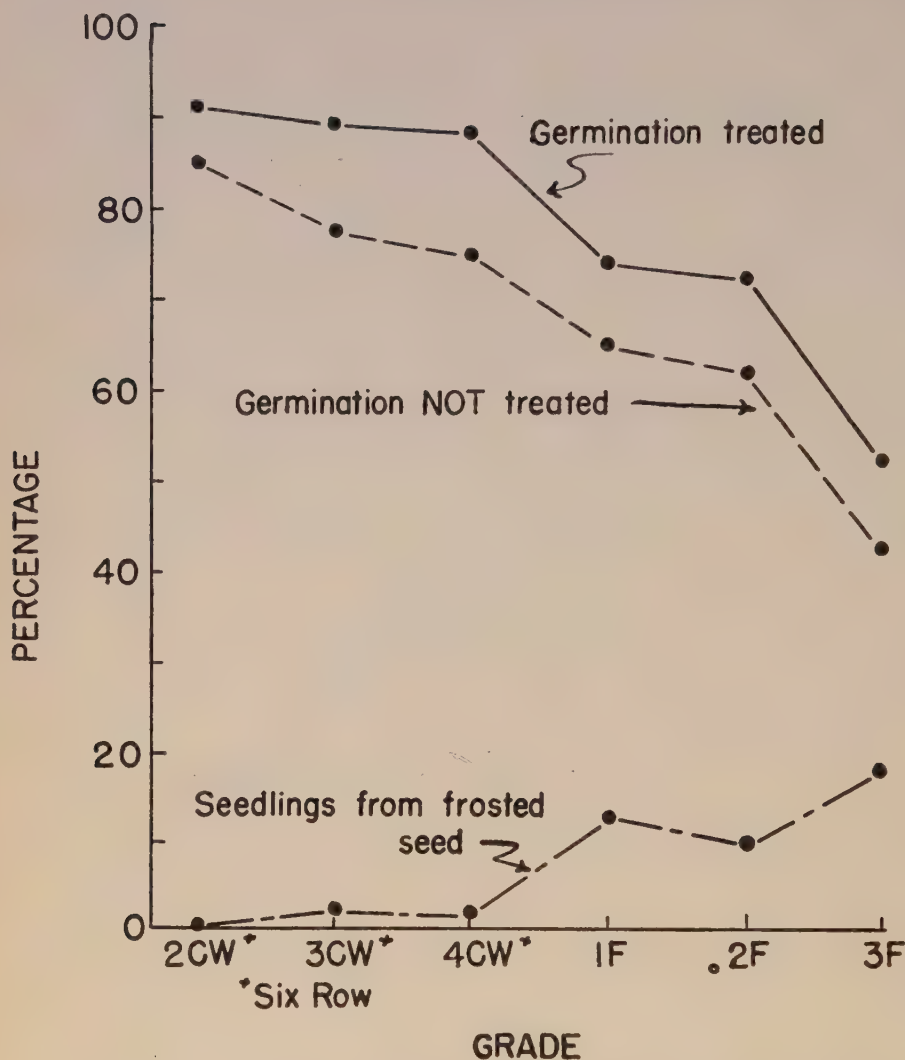


FIGURE 3. Germination (normal seedlings) of untreated and treated seed and the percentage of seedlings from seed injured by frost in different grades of barley.

well as the proportion of samples showing a 5 per cent or larger increase (Figure 2, Table 1), was less in the higher grades than in the lower. In barley (Figure 3, Table 1) the trend was indefinite, but it appeared that the intermediate grades responded to treatment more favourably than the highest and the lowest ones.

A study was made also of the effect of storage on the germination of treated seed of different grades. For this purpose 10 samples of each grade were taken at random, and half of each sample was treated and then stored in closed bottles, kept at room temperature, for 30 days. At the end of this period, the other half of each sample was treated and the halves were then sown in adjacent rows in a bed of soil in a greenhouse. A count



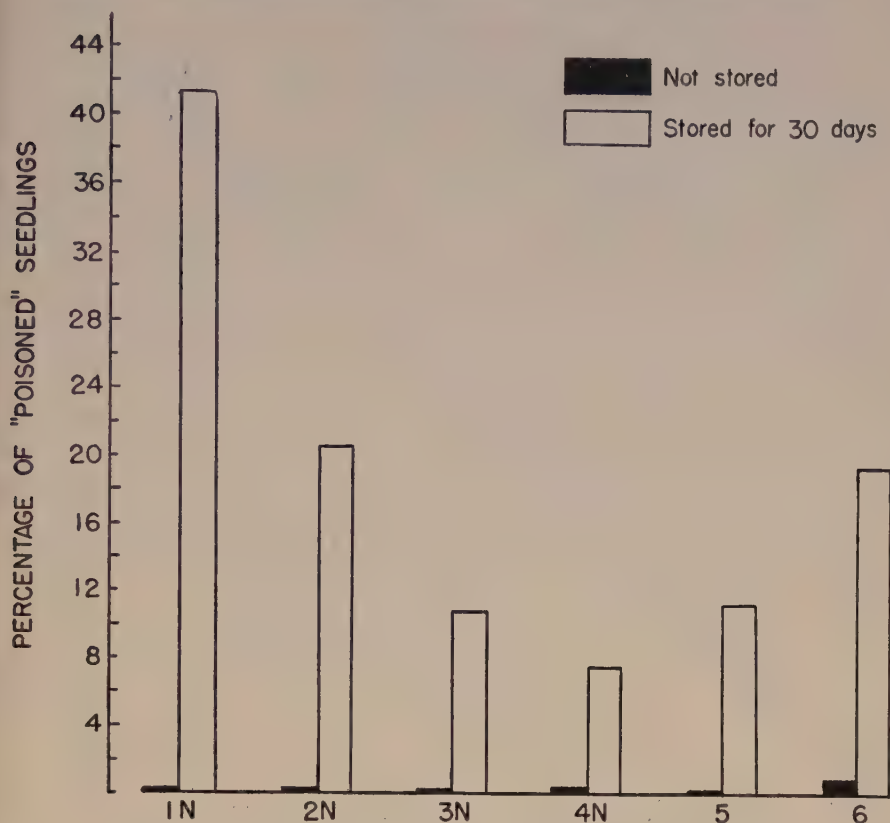


FIGURE 4. The percentage of "poisoned" seedlings from newly-treated seed and stored, treated seed for different grades of wheat.

of normal and abnormal seedlings was made in the usual way, a separate record being kept of the number of seedlings that showed the characteristic hypertrophy of the coleoptile resulting from "mercury poisoning".

Storage of treated seed decreased markedly both normal and total germination in wheat. It had but little effect on barley, and apparently no effect on oats. There was no relation between grade and reduction in germination in barley, but in wheat there was, the largest reduction occurring in No. 1 Northern and the least in No. 4 Northern. In samples graded below No. 4 Northern the adverse effect of storage became again more pronounced. Storage of treated wheat seed also had a marked effect on the numbers of seedlings showing "mercury poisoning", the newly-treated seed showing but little of this injury, while stored treated seed showed a great deal, particularly in the top and bottom grades (Figure 4). The amount of treatment injury in wheat seemed to be related to bushel weight (Figure 5), a correlation of the two variables showing the relation to be highly significant (coefficient = + 0.648). As might have been expected, most of the kernels in the top grades were plump. In the bottom grades also there were considerable numbers of plump kernels, but this grain had been excluded from the higher grades on account of frost injury.

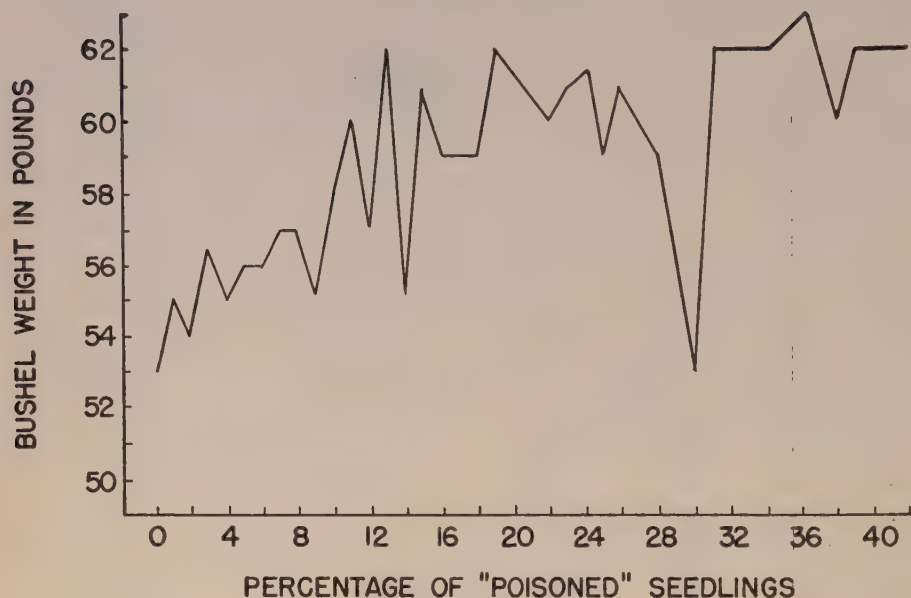


FIGURE 5. Relation of bushel weight to the percentage of "poisoned" seedlings in wheat seed treated and stored for 30 days.

The high proportion of hypertrophied seedlings in the top and bottom grades was probably due to threshing injury which resulted in considerable embryo exposure, especially among the large kernels. In the intermediate grades, where the grain was generally of low bushel weight, and the kernels were small or shrivelled, with little mechanical injury, the amount of coleoptile hypertrophy was negligible.

#### SUMMARY

A study was made of seed germinability, occurrence of frost injury, and the effect of seed treatment in several Canadian statutory and commercial grades of wheat, oats, and barley of the 1950 crop. It was found that germination generally decreased with grade, although the seed of a few samples of the lowest grades germinated as well as did that of the best samples of the highest grade. Injury from frost was found in all the grades studied, but the percentage of injured seeds increased as the grade became lower. Seed treatment increased germination in all grades, the improvement in wheat being more pronounced in the top grades than in the bottom grades, with the opposite trend in oats, and with little or no difference in barley. Storage of treated seed seriously reduced the germination of wheat in the top and bottom grades but only slightly in the intermediate grades. Storage had but little adverse effect on the germination of treated barley seed and had no effect on oats.

# LEACHATES FROM DECOMPOSING LEAVES

## I. SOME GENERAL CHARACTERISTICS<sup>1</sup>

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[Received for publication April 28, 1952]

Processes of soil formation are characterized by transportation and deposition of materials, both organic and inorganic, within the soil profile. The visible evidence of such movements is particularly apparent in the profiles of podsol and of certain podsollic soils, the striking colour spectra of which are due, primarily, to localization of accumulation of organic materials and of compounds of iron. The mechanisms involved in the translocation and localization processes which result in the observable profile variations in such characteristics as colour, degree of cementation, and concentration of iron compounds remain obscure. It seems probable, however, that, in the case of podsol soils formed under forest vegetation, substances leaching from the organic layer overlying the soil play an important role in the development of the characteristics distinguishing the profiles of such soils. Investigations, the objectives of which are to obtain information on the rate of decomposition of litter reaching the forest floor and on the characteristics of leachates produced from the litter by natural precipitation were begun in 1947. In a previous paper (1) data on the annual leaf-fall of some deciduous forest trees, on the composition of leaves collected as they fell, the composition of such leaves after partial decomposition, and on the properties of leachates collected from the leaves during decomposition, were presented. The purpose of the present communication is to provide further data respecting the nature of these leachates.

## EXPERIMENTAL MATERIALS AND METHODS

The procedure followed in gathering the leaves, and the apparatus and technique employed in the collection of leachates, have been described previously (1). The experimental arrangements for leaf exposure and leachate collection are illustrated in Figures 1 and 2.

Leaves of sugar maple were obtained from trees growing on Grenville (St. Bernard) soil, those of American beech and of poplar from Rubicon (St. Amable) soil, and those of gray birch from a soil of the Courval series in 1947; thereafter from Uplands sand. These soils have been described by Lajoie and Stobbe (2). They are classified as belonging to the following great soil groups; Grenville, brown forest; Rubicon, ground-water podsol; Courval, gray hydromorphic (dark gray gleisolic); Uplands, podsol.

The periods when collected and the conditions of exposure of the leaves are indicated in Table 1.

In the summers of 1950 and 1951 the decomposing leaves were inoculated with soil suspensions. This was done in an attempt to ensure the presence of a normal microbial flora. The inoculum was prepared as follows: Samples of soil to a depth of 2-3 inches were taken in or near the

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TABLE 1.—COLLECTION PERIODS, TREATMENTS AND DURATION OF EXPOSURE OF LEAVES

Leaf collection period	Treatment	Exposure period
Autumn 1947	Not inoculated	Eleven months, starting at end of leaf-fall period in 1947.
Autumn 1948	Not inoculated	Twenty-three months, starting at end of leaf-fall period in 1948.
Autumn 1949	Inoculated	Eighteen months, starting May 4, 1950.

TABLE 2.—WEIGHT OF LEAVES EXPOSED PER UNIT SURFACE AND PERCENTAGE LOSSES (D.W. BASIS)

Year collected	Beech		Birch		Maple		Poplar	
	Gm.	Loss	Gm.	Loss	Gm.	Loss	Gm.	Loss
		%		%		%		%
1947	268	18.2	502	38.2	298	42.7	241	24.5
1948	218	41.4	241	51.9	325	55.8	213	49.8
1949	213	41.8	108	—	336	49.7	104	—

TABLE 3.—VOLUMES OF LEACHATES (IN LITRES) COLLECTED FROM 1948 LEAVES IN 1949 AND 1950 DURING THE INTERVALS INDICATED

Collection interval	Beech	Birch	Maple	Poplar
November-December, 1948	63.3	67.4	67.7	70.2
April, 1949	35.7	38.2	38.7	39.0
May, 1949	17.6	21.4	21.8	21.5
June, 1949	63.1	71.9	69.6	70.0
July, 1949	17.4	18.1	18.4	18.7
August-September, 1949	78.8	85.6	88.2	78.3
May-June, 1950	49.6	51.6	46.7	49.1
July, 1950	80.3	90.6	81.5	86.6
August-September, 1950	86.9	97.2	97.0	98.5
Total volumes collected	492.7	542.0	529.6	531.9

collection sites for each species of leaves. These were mixed with about a gallon of water and allowed to stand for several hours. The supernatant suspension then was filtered through cheesecloth into a watering-can and sprinkled over the leaves. Inoculations were made monthly from May to October, inclusive, in 1950 and in July, August and September in 1951.

The weights of leaves (oven-dry basis) exposed per square foot of tray surface and the percentages of loss in weight occurring during the exposure periods (Table 1) are presented in Table 2.



FIGURE 1. Leaf exposure

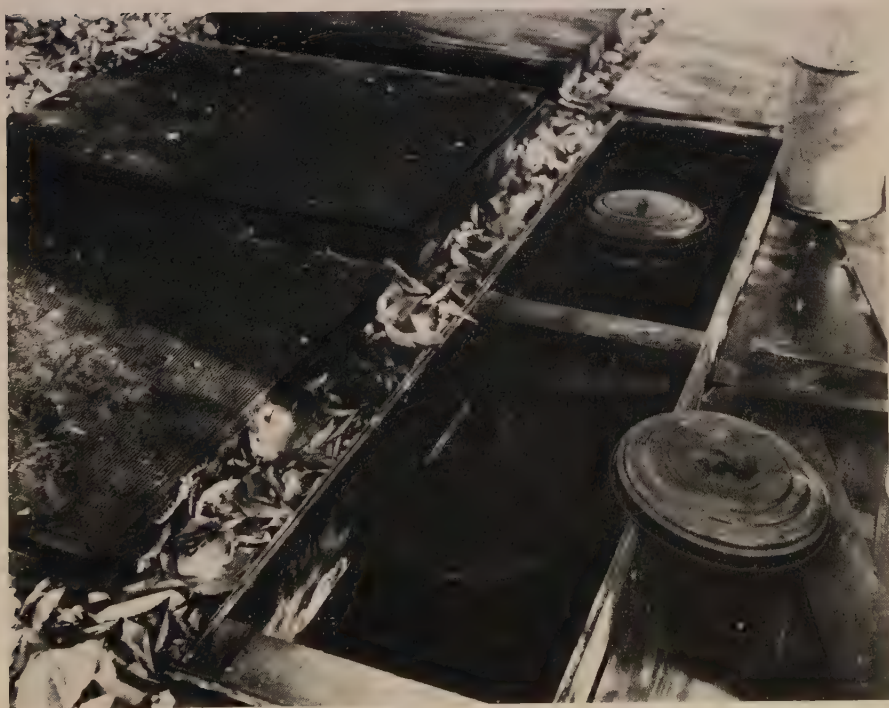


FIGURE 2. Leachate collection





The volumes of leachates gathered from the 1948 leaves during the leachate collection periods indicated are presented in Table 3. From these data and from the information given in the figures presented below, the total amounts of titratable acidity, total solids, ash, and ash alkalinity present in the leachates may be calculated.

With the exception of the winter months, leachates were collected after each rainfall of sufficient magnitude to cause percolation, filtered to remove suspended material, and the volumes obtained recorded. Routine determinations of the following characteristics were made on aliquots taken from each leachate collected:

1. pH, using a Beckman Model G potentiometer with glass-calomel electrodes;
2. Titratable acidity or alkalinity by titration with 0.01 N NaOH or HCl solution to pH 7;
3. Total solids and ash by the methods used for potable water (4); and
4. Ash alkalinity by the method of Purves (5).

Total nitrogen by the micro-Kjeldahl method (4) was determined on leachates collected in 1948 from leaves gathered in 1947, also on concentrates of leachates gathered on June 9, 1948. These concentrates were obtained by distillation and the degrees of concentration obtained were 55-, 25-, 50- and 50-fold, respectively for leachates from beech, birch, maple and poplar leaves of the 1947 collection. Free amino-nitrogen also was determined on these concentrates using the micro Van Slyke apparatus. In addition, the concentrates were centrifuged to remove suspended solids and aliquots of the supernatant solutions hydrolyzed for identification of the amino acids present. For hydrolysis an equal volume of concentrated HCl solution was added and the mixture refluxed for 24 hours in an all-glass apparatus. The HCl was removed from the hydrolysates by four evaporations to dryness under vacuum, a small amount of distilled water being added after each evaporation. The acid-free residues so obtained were analysed for amino acid content by paper partition chromatography (ascending technique). Identification of the amino acids was by means of  $R_f$  values and by comparison with standard solutions of amino acids of known identity.

Numerous collections of leachates were made, and since these were produced as a result of natural rainfall, these collections came at very irregular intervals. For convenience of presentation, therefore, the analytical data obtained have been calculated to a monthly or a bi-monthly basis. The pH values for leachates gathered during these periods have been averaged and the other data calculated as the mean values per unit volume of 10 litres. The information so obtained is presented in the form of curves suggestive of general trends in leachate characteristics with the progress of the season and of leaf decomposition. Detailed data will be presented for the 1948 leaves only. Deviations in characteristics shown by leachates collected from 1947 and from 1949 leaves, as compared with those of leachates obtained from 1948 leaves, will receive comment in instances in which such deviations seem to be large enough to have significance, even though the cause of the deviations be obscure.

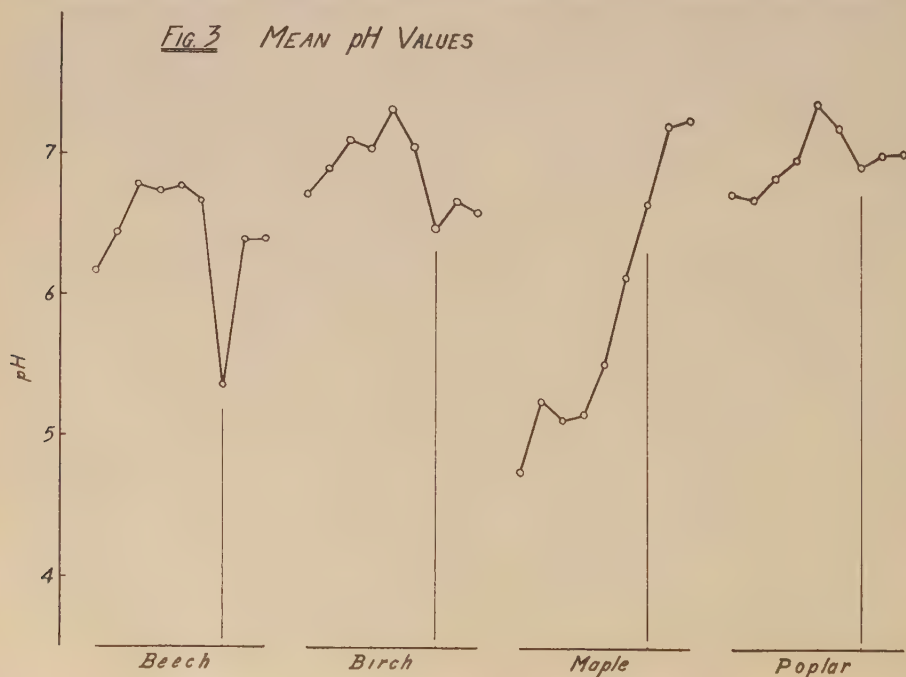
## EXPERIMENTAL RESULTS AND DISCUSSION

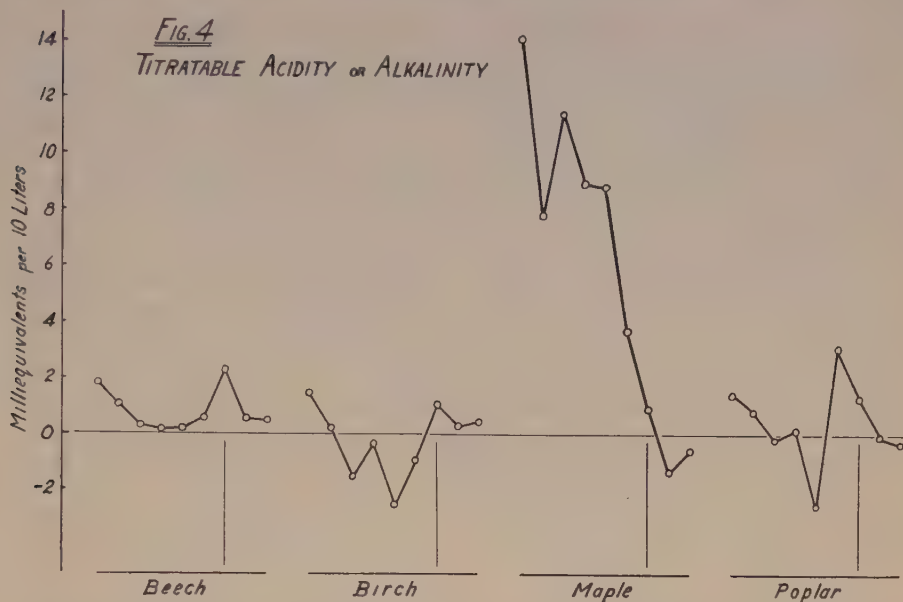
The pH trends for leachates from the four species of leaves (1948 collection) exposed during 1949 and 1950 are shown in Figure 3. In this and the subsequent figures the perpendicular lines beneath the curves indicate the values for leachates of the first collection interval of the second season of exposure. All values at the left of these lines are for leachate collections of the first season of exposure. The collection periods represented by the points plotted in Figures 3 to 6, inclusive, were as follows, reading from left to right in these figures; November-December, April, May, June, July, August-September, May-June, July and August-September. The analytical data for 1947 and for 1949 leaves, with which those given in Figures 3-6 are compared, were summarized in collection periods thus: for 1947 leaves, October-November, April, May, June, July and August-September, for 1949 leaves, May-June, July and August-September.

Examination of Figure 3 shows that, in each season of exposure, there is a general trend toward decreasing intensity of acidity of the leachates with advance of the season, this trend being much more marked in the first than in the second season. The great change occurring in successive collections of maple leachates is noteworthy.

The observation that the pH of leachates from decomposing leaves rises as decomposition progresses is in agreement with the earlier results of Stepanov (6, 7). It may be noted also that Marten and Pohlman (3) found that the pH values of decomposing leaves rose with advancing decomposition.

Secondly, it may be observed that, for the first season of exposure, the leachates from the different species of leaves may be arranged in order of





decreasing intensity of acidity, or pH, as follows: maple, beech, birch and poplar. Leachates from the latter two species are approximately equal in this respect. Those from poplar were the more acidic in the early part of this season and were slightly more alkaline for the last two collection intervals.

Leachates from 1947 leaves, exposed from autumn 1947 to autumn 1948, fell into nearly the same order of intensity of acidity, namely, maple, beech, poplar and birch, the four curves being distinctly different throughout the season of 1948. For 1949 leaves exposed from May to September (inclusive) 1950 the order was maple, birch, beech, poplar. No definite explanation of the changing order of species can be given. It is thought possible, however, that the differences in weight of leaves exposed per unit area of tray surface may have constituted an important factor in this connection, and that, for example, the large weight of 1947 birch leaves, as compared to that of 1949 leaves of this species exposed, may account for the greater alkalinity of leachates from the former.

It may be noted that variations in mass of decomposing leaves per unit surface area is a natural condition in the forest floor, especially when the vegetation is deciduous and the floor has marked degree of micro-relief.

During the second season of exposure the order of decreasing intensity of acidity was the same for both 1948 and 1949 leaves and different from that for the first season. It was beech greater than birch and poplar approximately equal to maple, the slowly decomposing beech leaves giving the most intensely acidic leachates in the second season of exposure. Differences in intensity of acidity in the second season were small as compared to those during the first season, as Figure 3 demonstrates.

In Figure 4 the changes in the titratable acidities of the leachates from 1948 leaves are shown. Inspection of this figure shows that, during the first season of exposure, maple leachates possessed by far the greatest

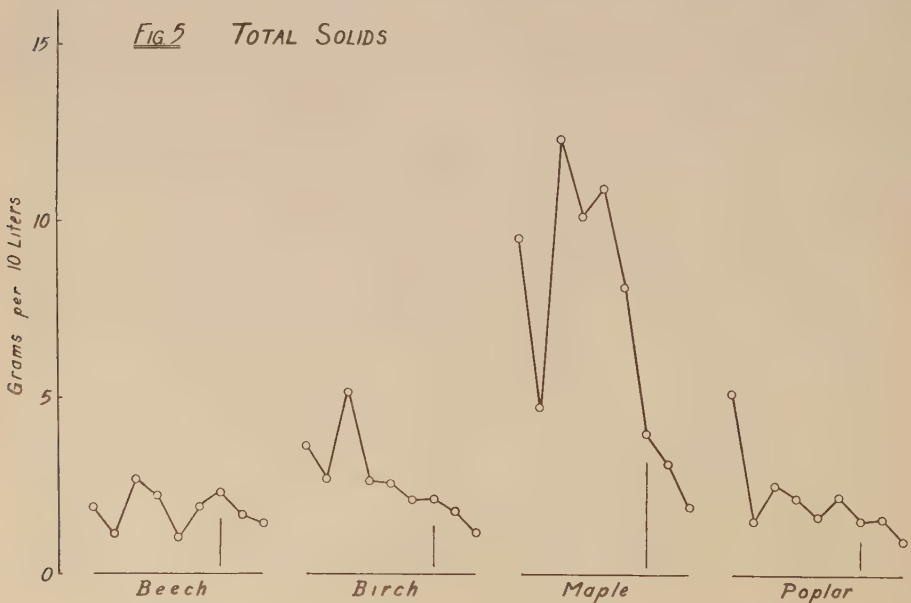


initial titratable acidity and exhibited the greatest over-all range in this characteristic. Beech leachates were next to those of maple in titratable acidity during this first season. In the initial season of exposure leachates obtained from 1947 and from 1949 leaves were similar in these respects. In the case of the 1947 leaves, however, birch leachates once more were different from those obtained from 1948 and 1949 leaves of this species in that they exhibited a much greater range of titratable acidity.

In the second season of exposure differences in the titratable acidities of leachates from different species were relatively small, and the acidities after the first collection period either were low or negative. In the second and third collection periods of this season, maple leachates tended to have the lowest acidities. This was true for both 1948 and 1949 leaves.

Of the four species studied, leachates from beech were the only ones which never gave a measurable amount of titratable alkalinity in either season of exposure. For both 1948 and 1949 leaves leachates from birch and poplar were found to possess titratable alkalinity (expressed as negative acidity in Figure 4) in the first season. The same was true for birch, but not for poplar leachates of the first season from 1947 leaves. In no case did maple leachates have titratable alkalinity in the first season of exposure. The sharp rise in the titratable acidity of poplar leachates at the end of the first season (Figure 4) was not shown in the case of the 1947 and 1949 poplar leaves.

The changes in the total solids contents of leachates from 1948 leaves are portrayed in Figure 5. These data show that first season maple leachates have by far the greatest total solids content per unit of volume, while first season beech leachates have the least. These remarks hold true also for leachates from 1949 leaves in the initial season of exposure. With 1947 leaves, however, the total solids content of leachates from birch leaves





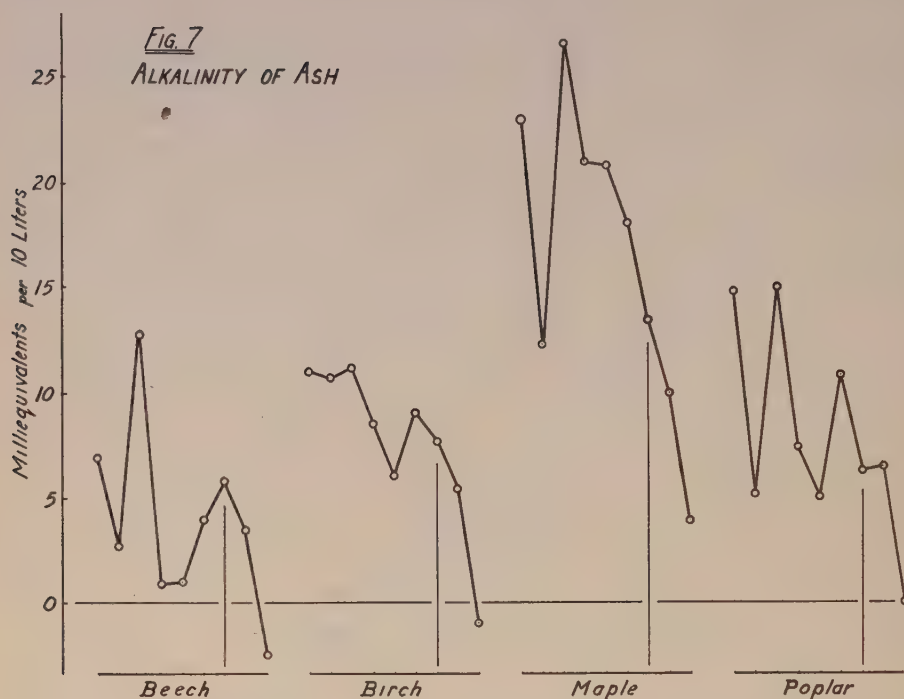
was greater than that of leachates from maple. This is thought to be a consequence of the relatively large weight of birch leaves per unit area of the tray exposed in the 1947-48 season.

In the second season of exposure the total solids contents of leachates from different leaf species were small and closely similar. Nevertheless, the solids contents of maple leachates still were higher than those for leachates from the other species.

Figure 6 presents the data obtained for the ash contents of the leachates from the 1948 leaves. In respect of this component, as well as of titratable acidity and total solids, leachates from maple and from beech leaves are seen from Figure 6 to represent the extremes, maple leachates having high and beech leachates low ash contents with birch and poplar intermediate. Comparison with the data for 1947 and 1949 leaves in the first season of exposure indicated that this order held for the 1949 leaves. In leachates from 1947 leaves, however, the order of decreasing ash content was birch, poplar, maple, beech. Possibly the large quantity of birch leaves exposed per unit of surface was once again responsible for the position of birch leachates in the series just given. On the other hand, the order of weights exposed per unit of surface is not that of the ash content of the leachates when all four species are considered. The differing behaviour of the leachates for 1947 leaves in respect of ash content remains unexplained. Possibly seasonal climatic conditions are important in this connection.

In general, the ash contents of the leachates run more or less parallel to their total solids contents. This relationship is most clearly shown by leachates obtained in the second season of exposure, maple leachates standing highest in both these respects, those from the other three species being closely similar and less than for maple for both 1948 and 1949 leaves.

The values obtained for the alkalinity of the ash of leachates obtained from 1948 leaves are given in Figure 7. Once more the familiar pattern of maple leachates high, beech leachates low, appears. This pattern holds



true also for both 1948 and 1949 leaves in both seasons of exposure. Leachates from 1947 leaves did not conform, birch leachates once more being out of the expected order and showing alkalinity greater than that of maple leachates. It may be noted also that the very sharp decline in ash alkalinity exhibited by all leachates from 1948 leaves in the second season of exposure was not shown by second season leachates from inoculated 1949 leaves.

In the opinion of the authors the most significant features of the data presented in Figures 3-7, from the standpoint of their relation to the podsolization process, are the relatively low pH values and the relatively high titratable acidities of the initial leachates obtained from the leaves. It seems noteworthy that, under field conditions, these initial leachates are produced in late autumn and early spring during periods of very low biological activity. Examination of Figures 3 and 4 shows that the pH values of the leachates increase and their titratable acidities decrease as conditions for microbial activity improve, and that these trends are reversed in late autumn and early spring. Further, these initial leachates, solutions of relatively high potential leaching capacity, are produced at periods when the moisture supply generally is abundant and the evaporation rate low, i.e., periods favourable for leaching.

In the opinion of the authors, however, too great emphasis should not be placed on the acidic characteristics of leaf leachates. Experimental work in progress indicates that acidity is not the only factor concerned in the movement of iron and aluminium by leachates from decomposing leaves, and that other characteristics may be equally or more important. It is recognized, moreover, that the biological conditions of the forest floor



are not being reproduced completely under the experimental conditions discussed. This, it is believed, is particularly true of the faunal effect in leaf decomposition. The influence of the fauna of the forest floor on the characteristics of the leachates produced would, however, be expected to be at a minimum during late fall and early spring.

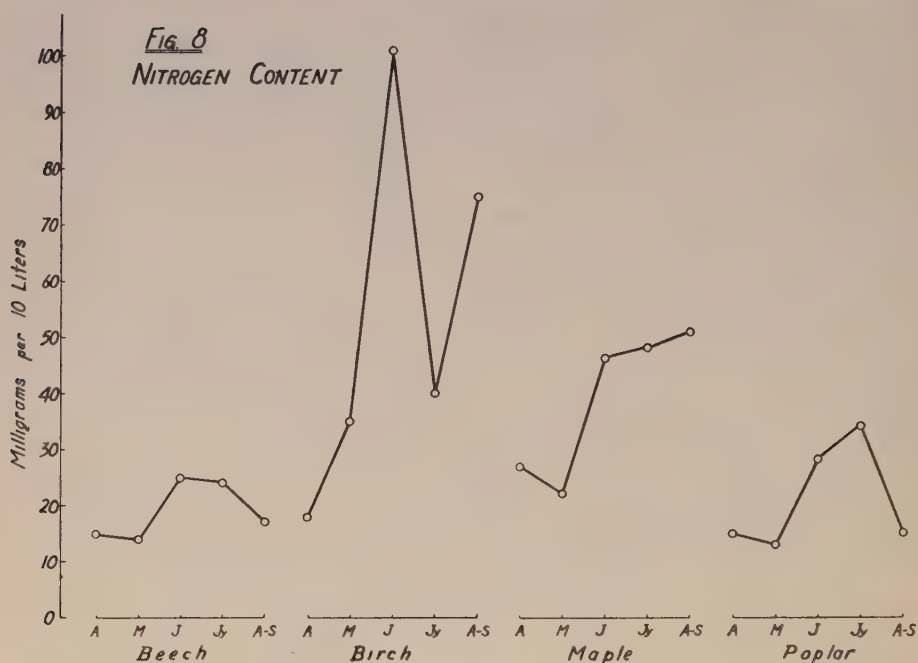
The significance of the differences exhibited by leachates from different species of leaves is not yet apparent. A factor complicating interpretation is the fact that the maple leaves used were produced by trees growing on a neutral to slightly alkaline soil formed from calcareous till, those of the other three species from acidic soil sites. The extent to which this difference in site conditions may be responsible for the differences in characteristics shown by leachates from maple leaves as compared to those from beech, birch and poplar leaves is unknown. Preparations are being made, however, for a comparison of maple leachates produced from leaves grown on the Grenville soil and from leaves grown on an acidic soil. It is hoped that the results obtained may assist in the interpretation of the data here presented.

The leachates obtained under the experimental conditions employed characteristically contained but small amounts of nitrogen. The data obtained from leachates collected during the summer of 1949 from leaves gathered in 1947 are presented in Figure 7, in terms of milligrams of total nitrogen found per 10 litres of leachate. The collection intervals represented in this figure are the months of April, May, June, July, and August-September indicated by A, M, J, Jy, and A-S, respectively. Inspection of this figure shows that the highest concentrations of nitrogen generally were attained in June or July, and that in all collection intervals, with the exception of the July period, the nitrogen concentration in birch leachates was greater than in those from the other species of leaves. The total amounts of nitrogen recovered in the whole collection period, April to August-September, inclusive, were, for beech, birch, maple and poplar, 0.45, 1.44, 0.98 and 0.51 grams, respectively. The nitrogen contents of the organic material contained in these leachates, that is, material not volatile on the steam bath but volatile on ignition, were found to be higher for beech and poplar than for birch and maple leachates. The mean values for percentage of nitrogen in the organic material in the leachates obtained during the 1948 season were 1.72, 0.90, 0.74 and 1.31 for beech, birch, maple and poplar leachates, respectively.

Leachates of the June 9, 1948 collection were concentrated by vacuum distillation and the total nitrogen and apparent amino-nitrogen contents of these concentrates were determined, the latter by the Van Slyke micro-manometric method. The results obtained are presented in Table 4. In

TABLE 4.—TOTAL NITROGEN AND AMINO-NITROGEN CONTENTS OF LEACHATE CONCENTRATES

Species	Total nitrogen	Amino-nitrogen	
	Mgm./l.	Mgm./l.	Per cent of total nitrogen
Beech	0.82	0.40	49
Birch	11.5	1.73	15
Maple	1.56	0.92	59
Poplar	0.56	0.32	57



this table the concentrations of total and of amino-nitrogen are given in terms of milligrams per litre of leachate.

Since it is well known that substances other than amino acids can yield free nitrogen under the conditions of the Van Slyke determination, the leachate concentrates were examined for the presence of amino acids using the paper partition chromatographic technique. The concentrates were centrifuged to remove suspended solid material before hydrolysis for estimation of amino acids. Identification of amino acids in the hydrolysates was by comparison with standard solutions of known amino acids and with  $R_f$  values recorded in the literature. In this manner tentative identification of the following amino acids was obtained, namely, (1) alanine, (2) aspartic acid, (3) glycine, (4) leucine (or isoleucine), (5) proline, and (6) valine (or norvaline). The distributions of these amino acids in the leachates from the four species of leaves were as follows: beech, 1, 2, 3, 6; birch, 1, 2, 3, 4, 6; maple, 1, 3, 6; poplar, 1, 3, 4, 5, 6. It is perhaps noteworthy that definite indications of the presence of alanine, glycine and valine (or norvaline) were obtained from leachates of all species of leaves examined.

### SUMMARY

Chemical characteristics of leachates obtained by exposure of leaves of four species of deciduous forest trees (beech, gray birch, sugar maple and poplar) on trays placed in the soil of the forest floor have been investigated. It has been found (a), that such leachates tend to become less acidic as the time of exposure lengthens, but that this trend may be reversed during the interval between two growing seasons; (b) that the pH values and the titratable acidities of leachates from sugar maple leaves collected

from a calcareous till soil approximately neutral in reaction are considerably lower and much greater respectively, than are the corresponding values for leachates from beech, birch and poplar leaves collected from acidic soil sites; (c) that the total solids and ash contents of leachates from the maple leaves also are greater than those of leachates from leaves of the other species; and (d) that the alkalinity of the ash of maple leachates also tends to be greater than that of leachates from the other species.

Observations on the nitrogenous fraction of the leachates have indicated that the total nitrogen content, always low, tended to be highest in June and July collections and that amino acids were present. Alanine, glycine and valine (or norvaline) were tentatively identified as present in leachate collections made in early June from all four species of leaves.

#### ACKNOWLEDGMENT

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# THE USE OF LATTICE DESIGNS FOR TESTING FORAGE CROPS<sup>1</sup>

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Lattice designs were introduced by Yates in 1936 for testing large numbers of strains (9). Considerable literature now has accumulated showing the suitability of these designs for testing annual crops. Less information is available regarding the efficiency of lattice designs for testing perennial crops. The purpose of this paper is to present efficiency ratings of tests employing lattice designs which have been carried out at Saskatoon from 1944 to 1951 on perennial forage crops.

## LITERATURE REVIEW

Lattice designs generally have resulted in considerable gains in efficiency over randomized block designs in testing annual crops. In corn trials Zuber (10) found the average efficiency of lattice designs to be 136 per cent of that of randomized block designs. Cochrane (3) reported efficiencies of 114 to 365 per cent for corn. Johnson and Murphy (4) gave relative efficiency ratings of 155 to 224 per cent for lattice and lattice square designs in oats. Ma and Harrington (5) found the average efficiency for lattice designs to be 148 per cent for cereal crops. Torrie *et al.* (7) and Boyce (2) showed lower gains of 9 and 18 per cent, respectively for lattice designs in cereal crops.

Of the various lattice designs used Ma and Harrington found the balanced lattice most efficient with a gain in precision of 98 per cent. Increased efficiencies for other designs were 28 per cent for the simple lattice, 60 per cent for the triple lattice, 63 per cent for the quadruple lattice, and 32 per cent for the lattice square. Johnson and Murphy (4) obtained a larger error variance for the simple lattice than for the triple lattice and the lowest error variance for the lattice square. Robinson *et al.* (6) in peanut trials found the triple lattice slightly less efficient than the balanced lattice and the simple lattice slightly less efficient than the triple lattice.

The precision of lattice designs relative to randomized block arrangements is subject to wide variation. As soil variability increases the use of lattice designs becomes more efficient (4). Although efficiency is often proportional to the number of strains tested (4, 6) good gains in precision may be obtained from designs with 9 or 16 strains (1, 8). Robinson *et al.* (6) also found gains in precision to increase as length and width of plot were increased.

## RESULTS

Efficiencies of lattice designs were found by dividing the effective error of the lattice into the randomized block error. Coefficients of variability were determined using the randomized block error variance. Table 1 presents a description of lattice tests conducted in crested wheatgrass,

<sup>1</sup> Contribution of the Division of Forage Crops, Experimental Farms Service, Canada Department of Agriculture.

<sup>2</sup> Agriculture Research Officer in charge of grass investigations.

TABLE 1.—EFFICIENCY OF LATTICE DESIGNS FOR FORAGE CROPS AS SHOWN BY TESTS OF CRESTED WHEATGRASS, BROMEGRASS, AND ALFALFA AT SASKATOON, SASK., 1944-1951

Test No.	Number of strains	Design	Plot size	Type of planting	Replicate size	Number of rep.	Yields	C.V.	Level of significance	Relative efficiency of lattice
<i>Crested Wheatgrass</i>										
1	16	Quadruple	30' X 5'	5 rows spaced 1 foot apart	80' X 30'	4	Hay 1948-50	14.8	1%	197
2	16	Quadruple	30' X 2'	Single row spaced 2 feet	32' X 30'	4	Hay 1948-50	15.2	1%	128
3	16	Quadruple	30' X 2'	Single row spaced 2 feet	32' X 30'	4	Hay 1948-50	24.4	1%	141
4	16	Triple rep.	20' X 5'	5 rows spaced 1 foot apart	40' X 40'	6	Hay 1949-51	11.0	1%	215
5	16	Triple rep.	20' X 2'	Single row spaced plants	32' X 20'	6	Seed 1951*	29.0	1%	103
6	36	Simple rep.	12' X 2'	Single row spaced plants	32' X 24'	4	Hay 1949-51	11.0	1%	116
7	36	Triple rep.	20' X 2'	Single rows 2 feet apart	32' X 40'	6	Hay 1946	24.0	1%	100
8	49	Quadruple	17' X 2'	Single row spaced plants	56' X 40'	4	Hay 1951	9.2	1%	114
9	49	Quadruple	17' X 2'	Single row spaced plants	56' X 40'	4	Hay 1944-46	15.5	1%	144
10	49	Quadruple	17' X 2'	Single row spaced plants	42' X 54'	4	Hay 1948	13.2	1%	119
11	49	Quadruple	17' X 2'	Single row 2 feet apart	56' X 34'	4	Hay 1948-50	9.6	1%	196
12	64	Quadruple	14' X 2'	Single row 2 feet apart	56' X 34'	4	Hay 1948-50	13.4	1%	219
13	64	Triple rep.	20' X 2'	Single row spaced plants	64' X 28'	4	Hay 1947-48	9.3	1%	136
					64' X 40'	6	Hay 1948	17.0	1%	140
				Average 13 tests—hay				14.4	—	151
<i>Bromegrass</i>										
14	9	Triple rep.	20' X 6'	6 rows spaced 1 foot apart	54' X 20'	6	Hay 1949-51	23.0	None	100
15	9	Triple rep.	20' X 3'	Single row spaced plants	27' X 20'	6	Seed 1951*	50.0	1%	120
16	16	Quadruple	15' X 6'	6 rows spaced 1 foot apart	48' X 30'	4	Hay 1950-51	8.3	1%	103
17	16	Triple rep.	20' X 3'	3 rows spaced 1 foot apart	48' X 20'	6	Seed 1946*	23.7	1%	147
18	25	Balanced	20' X 3'	3 rows spaced 1 foot apart	75' X 20'	6	Hay 1948-51	17.6	None	100
19	36	Triple rep.	20' X 3'	3 rows spaced 1 foot apart	54' X 40'	6	Hay 1951	18.9	5%	216
20	36	Triple rep.	20' X 3'	3 rows spaced 1 foot apart	54' X 40'	6	Hay 1950-51	13.3	5%	222
21	64	Triple	9' X 6'	12 rows spaced 6 inches apart	96' X 42'	3	Hay 1951	8.6	1%	115
22	64	Triple rep.	20' X 3'	Single row spaced plants	96' X 42'	6	Seed 1951	25.6	1%	100
23	64	Triple rep.	20' X 3'	Single row spaced plants	96' X 42'	6	Hay 1946	15.0	None	111
							Hay 1948	11.8	1%	211
				Average 10 tests—hay			Hay 1951	17.0	1%	144
								15.3	—	
<i>Alfalfa</i>										
24	36	Triple rep.	20' X 3'	3 rows spaced 1 foot apart	108' X 20'	6	Hay 1947-48	13.3	1%	138
25	36	Triple rep.	20' X 2'	Single rows spaced 2 feet apart	72' X 20'	6	Hay 1947-50	25.0	1%	138
26	144	Simple rep.	5' X 3'	Single row spaced 3 feet apart	216' X 25'	4	Seed 1944	37.2	1%	102
27	144	Simple rep.	10' X 3'	Single row spaced 3 feet apart	216' X 10'	4	Hay 1945	24.8	1%	131
							Seed 1944	25.9	1%	116
							Hay 1945	15.4	1%	150
				Average 4 tests—hay				19.6	—	139

\* Border rows cut for seed.



bromegrass, and alfalfa since 1944 when lattice designs were adopted at the laboratory. Analyses were based on total yields of tests for the period under observation.

As indicated in Table 1 lattice designs gave definite gains in precision relative to randomized block methods of analysis. Average gains in efficiency were 51, 44, and 39 per cent for tests of crested wheatgrass, bromegrass, and alfalfa, respectively. Within each crop there was considerable variation in precision of individual tests. There was no marked trend for lattice tests with lower number of strains to be less efficient. However, two tests of bromegrass with nine strains and one with 16 strains showed no gains in efficiency for lattice analysis of hay yields. Crested wheatgrass tests of 16 strains showed high efficiencies for lattice designs. It is difficult to compare various types of lattice designs used but a cursory examination of tests of crested wheatgrass indicates that quadruple lattices gave similar precision to that of triple lattices. It would appear on the basis of these trials that where 16 or more strains or treatments are being compared serious consideration should be given to the use of lattice designs.

Table 2 presents relative efficiencies of lattice designs for individual years of a selected number of lattice tests. There is much variation in efficiency from year to year but no definite trend for efficiencies to increase or decrease with age of stand is apparent. The high efficiency of test No. 25 in the third year was attributed to the lattice design eliminating growth differences resulting from the presence of a large snow drift down one side of the test in the spring of that year.

Replicate shape in most tests was kept as nearly square as convenience would allow. In tests numbers 1, 18, 24, 25, 26, and 27 replicate shape departed considerably from the square and for most of these trials high relative efficiencies for lattice designs were found. In effect, replicate shape for these tests was not satisfactory for efficient testing as randomized block designs. As perennial crops are subject to a variety of hazards such as seeding failures, winter killing, and disease, certain strains may fail and the randomized block analysis must be used. Replicate shape consequently should be kept reasonably square in case of the elimination of certain strains from the analysis.

Where large numbers of strains are to be tested in lattice designs the use of small plots is generally desirable. Frequently plot size is limited by the availability of small amounts of seed. A survey of tests included in Table 1 indicates that the levels of significance and coefficients of variability for trials employing small plots compare favourably with those tests using larger plots. The use of single rows spaced two feet apart for crested wheatgrass often gave lower coefficients of variability than did five-row plots or single rows of spaced plants. In bromegrass, tests of spaced plants and three-row plots gave coefficients of variability comparing favourably with larger plots. The use of single row plots for crested wheatgrass and the three-row plots for bromegrass has now become standard practice in preliminary strain testing at the Saskatoon laboratory.

Precision for lattice analyses of tests for seed varied from 102 to 147 per cent with an average precision of 117 per cent for the six seed tests. Seed production trials showed higher coefficients of variability than tests of hay. This may have resulted in part from harvesting border rows of



TABLE 2.—EFFICIENCIES OF LATTICE DESIGNS FOR DIFFERENT YEARS OF HARVESTING PERENNIAL CROPS

Test No.	Lattice efficiency relative to randomized block			
	First crop year	Second crop year	Third crop year	All years
1	232	100	149	197
2	149	128	104	129
4	165	130	153	215
5	102	118	—	116
8	157	144	—	144
10	198	100	227	196
24	108	152	—	138
25	107	109	217	138

plots which previously had been cut for hay. In test No. 20 of bromegrass, however, where seed was threshed from hay cut in advanced stages of growth, coefficients of variability were for the same cut areas. Coefficients of variability were 24.6 for seed and 8.6 for hay.

### SUMMARY

Analysis of 27 lattice tests of crested wheatgrass, bromegrass, and alfalfa for hay indicated average gains in efficiencies of 51, 44, and 39 per cent, respectively over randomized block analysis. While low efficiency gains were obtained for tests involving nine strains marked gains were noted for tests involving 16 or more strains. Precision varied widely for different years of cutting but no trend to lower or higher efficiency was noted as tests aged. Lattice designs were relatively more efficient than randomized block designs when replicates were long and narrow. However, the use of long narrow replicates in lattice designs would not appear desirable since randomized block methods of analysis may become necessary due to the elimination of certain strains by winter injury, disease, or other natural hazards. Where lattice designs included large numbers of strains the use of single row plots for crested wheatgrass and triple row plots for bromegrass appeared very satisfactory. A lower average gain in efficiency of 17 per cent for lattice designs was obtained for six tests of seed production.

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